

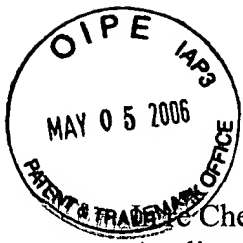
In re Chen, et al.

Application No. 09/854,208

Appeal Brief

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8.	CLAIM APPENDIX	Attached
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10.	RELATED PROCEEDINGS APPENDIX	None



Chen, et al.

Application No. 09/854,208

Appeal Brief

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application No.: 09/854,208  
Applicant: Chen, et al.  
Filed: May 10, 2001  
Group Art Unit: 1646  
Examiner: Dong Jiang  
Docket No.: 22338-403/P1381R1D1

**APPEAL BRIEF**

Mail Stop Appeal Brief-Patents  
Commissioner for Patents  
P.O. Box 1450  
Washington, D.C. 20231

Dear Sir:

A Notice of Appeal was filed on October 5, 2005. This brief is being filed with a petition for five-month extension of time and fee. Accordingly, Appellant submits that this Appeal Brief filed under 37 CFR § 41.37, is being timely filed on May 5, 2006. Appellant requests and authorizes the Commissioner to charge Deposit Account No. 18-1260 for the \$500.00 Appeal Brief fee due under 37 CFR 41.20(b)(2).

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**1. REAL PARTY IN INTEREST**

The real party in interest in this appeal is Genentech, Inc.

**2. RELATED APPEALS AND INTERFERENCES**

The Appellant, the Appellant's legal representative, or assignee are not aware of any appeals or interferences which may be related to, directly affect or be directly affected by or to have a bearing on the Board's decision in the pending appeal.

**3. STATUS OF CLAIMS**

Claims 1-42 were originally filed. Claims 43-59 were added and original claims 1-42 were cancelled at the time of filing in a preliminary amendment. Claims 43-59 were cancelled and new claims 60-70 were added in response to a restriction requirement on August 12, 2002. Claims 60-65 were cancelled, claims 67-69 were amended, and new claims 71-82 were added pursuant to a response and amendment filed on October 29, 2002. Appellant further amended claims 69, 71, 74-76 and 79-80 on January 23, 2003. The claims involved in this appeal, claims 66-82, are presented in the claim appendix attached hereto.

**4. STATUS OF AMENDMENTS**

There are no outstanding amendments.

**5. SUMMARY OF CLAIMED SUBJECT MATTER**

The subject matter of the claims under appeal pertain to, *inter alia*, the following:

- In one aspect, the present invention relates to a composition of matter comprising a polypeptide selected from the group consisting of (a) a PRO1122 polypeptide comprising amino acid residues 1 to 197 of SEQ ID NO:3, and (b) a PRO1122 polypeptide comprising amino acid residues 19 to 197 of SEQ ID NO:3; in combination with a

pharmaceutically acceptable carrier. *See, e.g.*, SEQ ID NO:3, Figures 3, 9, and 10, page 3, line 16 to page 4, line 9, page 7, lines 9-27, page 9, lines 14-33, page 11, lines 3-16, page 11, lines 27-36, page 12, lines 13-17, pages 27-34, page 67, line 26 to page 70, line 8, page 71, line 22 to page 72, line 4, Examples 4-6 and 10.

- In another aspect, the present invention relates to an article of manufacturing comprising: a container; and a composition of matter comprising an isolated polypeptide having at least 95% amino acid sequence identity to: (a) amino acid residues 1 to 197 of SEQ ID NO:3, (b) amino acid residues 19 to 197 of SEQ ID NO:3, (c) the amino acid encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203522, or (d) the amino acids encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203522 lacking its associated signal peptide encoding region; wherein said isolated polypeptide is capable of inducing the production of TNF- $\alpha$  in human leukemia monocytic THP-1 cells. *See, e.g.*, SEQ ID NO:3, Figures 3, 9, and 10, page 8, line 30 to page 11, line 16, page 11, lines 27-36, page 12, lines 13-17, pages 27-34, page 67, line 26 to page 70, line 8, page 71, line 22 to page 72, line 4, Examples 4-6 and 10.
- In another aspect, the present invention relates to an isolated polypeptide comprising at least 95% amino acid sequence identity to: (a) the amino acid residues 1 to 197 of SEQ ID NO:3, (b) the amino acid residues 19 to 197 of SEQ ID NO:3, (c) the amino acids encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203522 lacking its associated signal peptide encoding region; wherein said isolated polypeptide is capable of inducing the production of TNF - $\alpha$  in human leukemia monocytic THP-1 cells. *See, e.g.*, SEQ ID NO:3, Figures 3, 9, and 10, page 8, line 30 to page 11, line 16, page 11, lines 27-36, page 12, lines 13-17, pages 27-34, page 67, line 26 to page 70, line 8, page 71, line 22 to page 72, line 4, Examples 4-6 and 10.
- An isolated polypeptide comprising: (a) amino acid residues 1 to 197 of SEQ ID NO:3, (b) amino acid residues 19 to 197 of SEQ ID NO:3, (c) the amino acids encoded by the



full-length coding sequence of the cDNA deposited under ATCC accession number 203522, or (d) the amino acids encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203522 lacking its associated signal peptide encoding region. *See, e.g.*, SEQ ID NO:3, Figures 3, 9, and 10, page 8, line 30 to page 11, line 16, page 11, lines 27-36, page 12, lines 13-17, pages 27-34, page 67, line 26 to page 70, line 8, page 71, line 22 to page 72, line 4, Examples 4-6 and 10.

## **6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL**

Whether the rejection of pending claims 66-82 under § 102(e) as being anticipated by Ebner et al., U.S. 2003/0092133 (hereinafter referred to as “Ebner”), is proper.

## **7. ARGUMENT**

Throughout prosecution of the present application, the Examiner has maintained that Ebner discloses a polypeptide of IL-21, which has the amino acid sequence of SEQ ID NO:29, and is 100% identical to SEQ ID NO:3 of the present invention. The Examiner has also asserted that Ebner teaches a pharmaceutical composition comprising the polypeptide and a kit comprising the composition. The Examiner has taken the position that functional limitation recited in the presently rejected claims, of inducing the production of TNF- $\alpha$  in THP-1 cells and for treatment of a degenerative cartilaginous disorder are either an inherent property of the same composition or an intended use of the claimed composition and do not alter the nature of the composition. The Examiner also notes that the informational content of the printed matter is given no weight and does not render the article patentable distinct from the teachings of Ebner. Furthermore, the Examiner asserts that Ebner teaches a fusion protein comprising said polypeptide fused to antibody domains such as an antibody Fc region. See February 24, 2003 Office Action, page 3 (Paper no. 16).

The Examiner's position appears to be that by merely disclosing a nucleotide sequence, without disclosing any information regarding any biological role, function, or activity of either the nucleotide sequence or the polypeptide encoded thereby, the Ebner disclosure fulfills the

requirements for anticipation of the present claims under 35 U.S.C. § 102(e). Appellant disagrees with this conclusion of law. In particular, as set forth in previous responses, Appellant maintains that in order to be given effect under § 102(e), the claims of the reference patent or published patent application must be supported in the manner required by 35 U.S.C. § 112 in the priority application whose date is relied upon to establish the prior art status of the patent. *See In re Wertheim*, 646 F.2d 527, 209 USPQ 554 (CCPA 1981); and MPEP 2136.03, sub-heading IV. If the priority application fails to support the claims at issue (i.e., being rejected) in the manner required by § 112, the reference is not entitled to have prior art effect under § 102(e) as of the filing date of that priority application. In the present case, Ebner claims benefit to, *inter alia*, three provisional applications that were filed prior to the May 15, 1999 effective filing date the examiner recognized for the instant application.<sup>1</sup> However, the provisional applications to which Ebner claims benefit, U.S. Provisional Application No. 60/087,340 (“the ‘340 provisional”), 60/099,805 (“the ‘805 provisional”), and 60/131,965 (“the ‘965 provisional”), do not provide a specific, substantial and credible utility for the claimed subject matter. In fact, Appellant notes that the PTO has denied Ebner the benefit of priority to all of its earlier-filed applications, including the ‘340, ‘805 and ‘965 provisionals for failing to satisfy § 112 requirements. *See* Office Action dated January 24, 2006 in the application corresponding to the Ebner publication, U.S.S.N. 10/153,770. *See also* the February 10, 2004 Office Action wherein the Examiner acknowledged that Ebner was not entitled to the filing date of the ‘340 provisional because that application failed to disclose the polypeptide corresponding to the claimed invention. Appellant contends that because the provisionals do not satisfy the “how to use” prong of the § 112 requirements, Ebner is not entitled to the filing date of the provisional applications and cannot be prior art under § 102(e) to the Appealed claims.

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<sup>1</sup> In the Office Action dated 09/20/2002 (Paper No. 13), the Examiner determined that the instant application was entitled to the benefit of U.S.S.N. 09/311,832 filed on May 15, 1999. While the Appellant does not agree that May 15, 1999 is the earliest filing date to which the claims of the instant application are entitled, Appellant does not contest the Examiner’s benefit determination for purposes of this appeal. Appellant expressly reserves the right to contest such determination in the future as necessary.

The Ebner Publication is not prior art under § 102(e) in view of the inadequacies of the priority applications to which Ebner claims benefit under §§ 120 and 119(e)

Appellant observes the following deficiencies of the Ebner disclosure:<sup>2</sup>

- The Ebner disclosure presents only questionable and uncertain homology-based predictions. Ebner notes that using BLAST and Megalign analysis, the amino acid sequences identified in their disclosure were each found to be “highly homologous” to several members of the Interleukin family. Particularly, the disclosure notes that SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:29 as disclosed in the ‘965 and ‘805 provisional applications contain at least four domains homologous to the translational products of the human mRNA for Interleukin (IL)-20, IL-17, the murine mRNA for IL-17 (mIL-17), and the human viral mRNA for IL-17 (vIL-17). The disclosure also notes that the full length sequence (SEQ ID NO:29) of the identified IL-21 molecule shares a “high degree” of amino acid sequence identity with IL-20, IL-17, mIL-17, and vIL-17 in three conserved domains. From this, Ebner suggests that “[b]ecause each of these IL-17 and IL-17- like molecules is thought to be important immunoregulatory molecules, the homology between these IL-17 and IL-17-like molecules and IL-21 and IL-22 suggest that IL-21 and IL-22 may also be important immunoregulatory molecules.” (See ‘805 provisional, pages 10-11). While Ebner provides an amino acid sequence comparison of the IL-21 protein with the known sequences of IL-17 and related molecules (See ‘805 provisional, Figures 3A and 3B), it does not state the percent identity that is shared.

Ebner thus presents a highly questionable homology analysis – both with respect to the degree and nature of the homology to IL-17. Appellant’s own analysis reveals

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<sup>2</sup> Appellant notes that the ‘805 and ‘965 applications provide nearly identical disclosure regarding the subject matter at issue (referred to as IL-21 by Ebner). For example, the experimental characterization of the claimed subject matter is the same between the ‘965 and ‘805 provisional applications. Accordingly, any assertion regarding the ‘805 provisional is illustrative of the Ebner disclosure, in general, and fully applicable to the ‘965 provisional as well.

that the polypeptide resulting from the identified nucleotide sequence shares only a 26-28% amino acid identity with IL-17. (See page 48 and Figure 7A of the present application). Ebner acknowledges that IL-17 exhibits pleiotropic biological activities on various cell types, including its role in stimulating the expression of other cytokines such as IL-6, IL-8, G-CSF, Prostaglandin E (PGE<sub>2</sub>), and Intercellular Adhesion Molecule (ICAM)-1. (See '805 provisional, page 2). However, there is nothing in Ebner to indicate that the biological activities of IL-17 will be shared by the claimed polypeptide, especially in view of the low degree of amino acid sequence identity shared between the molecules. Thus, Ebner's observed degree of sequence homology to IL-17 and other molecules conveys no meaningful information concerning the biological role, function or activity of the claimed polynucleotide and polypeptide sequences.

- Ebner does not disclose a substantial utility that is specific to the particular polynucleotide or polypeptide corresponding to their identified sequences. The Ebner disclosure states that the polynucleotides in question can be used for chromosome mapping, to control gene expression through triple helix formation or antisense DNA or RNA, for DNA-based identification techniques, and "at the very least" as molecule weight markers, as diagnostic probes, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response (see '805 provisional, pages 48-52). These applications and techniques, however, are not specific to the polynucleotides disclosed by Ebner. Indeed, the same uses can be stated to exist for *any* polynucleotide sequence. Similarly, Ebner's disclosed uses for the disclosed polypeptides -- such as to assay protein levels, to treat some unknown disease to replace absent or decreased levels of the polypeptide, as molecular weight markets, or to raise antibodies to the polypeptide -- are generally applicable to *any* polypeptide molecule (see '805 provisional, pages 52-54).

- The biological activities and disease states that Ebner identifies with the claimed polynucleotides and polypeptides are merely speculative and nonspecific. Among the supposed “biological activities” of IL-21, Ebner discloses that the protein “modulates” IL-6 secretion from NIH-3T3 cells. The reference, however, makes no indication about whether IL-6 amounts increase or decrease – or remain unchanged – as a result. Instead, the disclosure merely proposes the use of a known assay to determine the level of IL-21-mediated IL-6 secretion. A similar assay is referenced to determine whether IL-21 modulates lymphocyte proliferation without any indication as to the actual effect of these proteins. (See ‘805 provisional, pages 54-55). The same holds true for the other activities disclosed by Ebner, including the immune activity, chemotactic activity, and binding activity, that may (or may not) be associated with the protein. (See, e.g., ‘805 provisional, pages 56-58 and 61-63). These disclosures of biological activity are merely speculative, unsupported by biological data, and scientifically meaningless to one of skill in the art. Appellant notes in this regard that the various assays that Ebner proposes in their examples are completely prophetic and contain no actual biological data relating to the IL-21 protein (See ‘805 provisional, Examples 13-20). To the extent that Ebner provides any tissue distribution data through Northern blot analysis, it shows that the IL-21 protein is expressed in variety of normal tissues (i.e., thymus, adrenal cortex, spleen pancreas, lymph node, PBL, fetal liver, adrenal medulla, thyroid, small intestine, stomach, and heart) with no known correlation to particular disease states (See ‘805 provisional, page 66). This disclosure does not suggest or teach that the IL-21 protein can be used in any useful manner for diagnosis or treatment.
- Ebner merely speculates that IL-21 can be used in the treatment of a long and varied list of conditions from inflammation to cancer to AIDS. No support is provided in Ebner for the suggestion that the IL-21 polypeptide or antibodies to them can be used in the variety (dozens) of therapeutic applications recited (See, e.g., ‘805 provisional, pages 56-60). Indeed, Ebner appears to have based this prediction on information

known about *other* interleukin family members, *not* the particular IL-21 polypeptide disclosed. If anything, the recitation of such a laundry list of varied and biologically diverse conditions suggest that Ebner in fact had no idea of what IL-21 could actually be used for.

Thus, while Ebner discloses certain sequence structure information for the IL-21 molecule, it utterly fails to disclose any information that would credibly describe any biological role, function or activity of the particular polypeptide. Instead, the disclosure speculates as to possible functions or activities that they might possess, but in a way that is so generalized and abstract as to be meaningless to a person of ordinary skill in the art. Indeed, there is no data provided in the Ebner disclosure that can reasonably establish *any* biological function or activity of the putative cytokine, much less information that could establish a specific role of the nucleic acid, polypeptide, or its antibodies. Such a disclosure, which provides no experimental data establishing a biological role for the polynucleotide and polypeptide, is scientifically meaningless to a person of skill of the art.

Appellant maintains that the scientifically deficient disclosure of Ebner renders it legally insufficient under § 102(e) to anticipate the rejected claims. In particular, Ebner's disclosure does not provide an accurate or unequivocal characterization of any biological function, activity or role of the claimed nucleic acid molecule. Instead, it merely discloses a polynucleotide sequence and speculates as to its biological function, role and activities.

Appellant submits that the proper legal standard for measuring the sufficiency of a U.S. patent for anticipation under 35 U.S.C. § 102(e) is that articulated by the Court of Customs and Patent Appeals in *In re Wertheim and Mishkin*, 209 USPQ 554 (CCPA 1981); namely, that a U.S. patent (in this case, patent publication) can anticipate under 35 U.S.C. § 102(e) as of a particular date only to the extent that there is a sufficient disclosure under 35 U.S.C. § 112, first paragraph, for the subject matter at issue (i.e., the subject matter of the claims being rejected as anticipated under §102(e) by the patent).

In *Wertheim*, the court addressed the specific question of the effective date – for prior art purposes – of a patent under 35 U.S.C. §§102(e) where the patent claimed the benefit of an earlier application under 35 U.S.C. §120. The court found that the §102(e) effective date of the patent was limited to that subject matter in the patent that could satisfy the requirements of 35 U.S.C. § 112, first paragraph, relative to the claims being rejected. Thus, the court recognized that a patent should be entitled to a prior art effect under § 102(e) *only as to subject matter that was disclosed in a manner that would be sufficient under § 112, first paragraph*. *Wertheim* states at 539 that “... the application, the filing date of which is needed to make a rejection, must disclose, pursuant to §§120/112, the invention claimed in the reference patent.” If the priority application fails to provide a sufficient disclosure, it may not serve as the basis for establishing a prior art effective date under § 102(e) for the patent or published application.

It is also well established that enablement requirement of § 112 incorporates the utility requirement of § 101. See *In re Ziegler*, 992 F.2d 1197, 1200-01 (Fed. Cir. 1993); MPEP § 2107.01. Therefore, if a reference provides no specific, substantial and credible utility for the claimed subject matter, it fails to satisfy the requirements of § 112 and cannot properly be considered as prior art under § 102(e). In this case, application of the PTO’s own utility guidelines clearly shows that the earlier-filed Ebner disclosures (along with Ebner itself) cannot support the present claims because they are incapable of establishing a specific, substantial and credible utility for the presently claimed subject matter. While there is no *per se* rule regarding homology-based assertions of utility, the Guidelines direct Examiners to take into account both the nature and the degree of homology recited in the application. Appellant notes the very low degree of homology (no more than about 28%) between the claimed polypeptide, and IL-17 and related cytokines. Even the minimal amount of homology that is provided is simply the result of data mining and cannot be used to confirm whether the proteins share any biological activities. No actual comparison of the structure or biological activities of the proteins is provided. Ebner thus cannot rely on its insufficient (both in terms of the degree and nature) homology analysis, particularly in the absence of experimental data characterizing the polypeptide at issue to

confirm its predictions, in order to establish a specific, substantial and credible utility for a putative nucleic acid molecule which encodes said polypeptide.

Additionally for the reasons noted, the asserted uses, biological activities, and therapeutic applications disclosed by Ebner are insufficient under § 101 because they qualify as neither “specific” nor “substantial” utility under Federal Circuit precedent. This principle was most recently reaffirmed in *In re Fisher*, 76 USPQ.2d, 421 F.3d 1365 (Fed. Cir. 2005), where the Federal Circuit held that claims to nucleic acid sequences for which the corresponding biological function is unknown fail to satisfy the utility requirements. The only known uses disclosed by Ebner are nonspecific ones such as “gene probe” and “chromosome marker,” which the court specifically found insufficient to qualify as utility in *Fisher*. As noted by the court, these represent “mere ‘objects of use-testing,’ to wit, objects upon which scientific research could be performed with no assurance that anything useful will be discovered in the end.” *Fisher* at 1231 (citing *Brenner v. Manson*, 383 U.S. 519, 535 (1966)). Furthermore, the biological activities and disease states that Ebner associates with the claimed polypeptides and polynucleotides are merely speculative and cannot establish utility. Ebner does not definitively associate any particular activity to the polypeptide or polynucleotide sequences that are disclosed and certainly provides no *in vitro* or *in vivo* data to support its speculated utility.

The insufficient disclosure in Ebner (i.e., the disclosure does not set forth a specific, substantial and credible utility for the nucleotide sequence and associated polypeptide), makes it impossible for the reference to satisfy the “how to use” prong of § 112 for the presently claimed subject matter. As a matter of law, then, because Ebner fails to satisfy the requirements of § 101, it fails to provide an enabling disclosure under § 112 and cannot anticipate the rejected claims under § 102(e).

In light of the arguments made above, it is respectfully submitted that the § 102(e) rejection over Ebner should be withdrawn and the pending claims passed to issue.



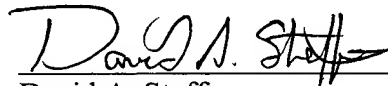
In re Chen, et al.  
Application No. 09/854,208  
Appeal Brief

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Appellant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 18-1260** referencing docket no. 22338-403. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: May 5, 2006

By:



David A. Steffes  
Registration No. 46,042  
Attorney for Appellant

Sidley Austin LLP  
1501 K Street, NW  
Washington, DC 20005  
Phone: 202-736-8157  
Fax: 202-736-8711

**8. CLAIM APPENDIX**

66. (previously presented) A composition of matter comprising a polypeptide selected from the group consisting of:

(a) a PRO1122 polypeptide comprising amino acid residues 1 to 197 of SEQ ID NO:3, and

(b) a PRO1122 polypeptide comprising amino acid residues 19 to 197 of SEQ ID NO:3; in combination with a pharmaceutically acceptable carrier.

67. (previously presented) The composition of matter of Claim 66 comprising a PRO1122 polypeptide comprising amino acid residues 1 to 197 of SEQ ID NO:3 in combination with a pharmaceutically acceptable carrier.

68. (previously presented) The composition of matter of Claim 66 which comprises a PRO1122 polypeptide comprising amino acid residues 19 to 197 of SEQ ID NO:3 in combination with a pharmaceutically acceptable carrier.

69. (previously presented) An article of manufacturing comprising:

a container; and

a composition of matter comprising an isolated polypeptide having at least 95% amino acid sequence identity to:

(a) amino acid residues 1 to 197 of SEQ ID NO:3,

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Appeal Brief

(b) amino acid residues 19 to 197 of SEQ ID NO:3,

(c) the amino acid encoded by the full-length coding sequence of the cDNA

deposited under ATCC accession number 203522, or

(d) the amino acids encoded by the full-length coding sequence of the cDNA

deposited under ATCC accession number 203522 lacking its associated signal peptide encoding region; wherein said isolated polypeptide is capable of inducing the production of TNF- $\alpha$  in human leukemia monocytic THP-1 cells.

70. (previously presented) The article of manufacture of Claim 69, further comprising a label affixed to said container, or a package insert included in said container, referring to the use of said composition for the treatment of a degenerative cartilaginous disorder in a mammal.

71. (previously presented) An isolated polypeptide comprising at least 95% amino acid sequence identity to:

(a) the amino acid residues 1 to 197 of SEQ ID NO:3,

(b) the amino acid residues 19 to 197 of SEQ ID NO:3,

(c) the amino acids encoded by the full-length coding sequence of the cDNA

deposited under ATCC accession number 203522 lacking its associated signal peptide encoding region; wherein said isolated polypeptide is capable of inducing the production of TNF - $\alpha$  in human leukemia monocytic THP-1 cells.

72. (previously presented) The isolated polypeptide of Claim 71 which comprises the amino acid residues 1 to 197 of SEQ ID NO:3.

73. (previously presented) The isolated polypeptide of Claim 71 which comprises the amino acid residues 19 to 177 of SEQ ID NO: 3.

74. (previously presented) The isolated polypeptide of Claim 71 which comprises the amino acids encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203522.

75. (previously presented) The isolated polypeptide of Claim 71 which comprises the amino acids encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203522 lacking its associated signal peptide encoding region.

76. (previously presented) An isolated polypeptide comprising:

- (a) amino acid residues 1 to 197 of SEQ ID NO:3,
- (b) amino acid residues 19 to 197 of SEQ ID NO:3,
- (c) the amino acids encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203522, or
- (d) the amino acids encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203522 lacking its associated signal peptide encoding region.

77. (previously presented) The isolated polypeptide of Claim 76 comprising amino acid residues 1 to 197 of SEQ ID NO:3.

78. (previously presented) The isolated polypeptide of Claim 76 comprising amino acid residues 19 to 197 of SEQ ID NO:3.

79. (previously presented) The isolated polypeptide of Claim 76 comprising the amino acids encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203522.

80. (previously presented) The isolated polypeptide of Claim 76 comprising the amino acids encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203522 lacking its associated signal peptide encoding region.

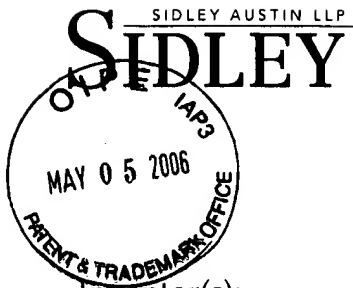
81. (previously presented) A chimeric polypeptide comprising a polypeptide according to Claim 71 fused to a heterologous polypeptide.

82. (previously presented) The chimeric polypeptide of Claim 81, wherein said heterologous polypeptide is an epitope tag or an Fc region of an immunoglobulin.

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## **9. EVIDENCE APPENDIX**

- U.S. Provisional Application No. 60/099,805;
- U.S. Provisional Application No. 60/131,965; and
- Office Action dated January 24, 2006 for U.S.S.N. 10/153,770 (corresponding to Ebner publication No. 2003/0092133).



SIDLEY AUSTIN LLP  
1501 K STREET, N.W.  
WASHINGTON, D.C. 20005  
(202) 736 8000  
(202) 736 8711 FAX

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### PTO FILING/DOCKET RECEIPT

Inventor(s): Jian CHEN, et al.

Attorney Docket No. 22338-403

Serial or Patent No.: 09/854,208

Filing or Issue Date: May 10, 2001

Confirmation No. (if assigned): 8512

Title: IL-17 HOMOLOGOUS POLYPEPTIDES AND USES THEREOF

Please acknowledge receipt of the following:

1. Five-Month Extension of Time
2. Appeal Brief
3. Claim Appendix
4. Evidence Appendix containing
  - (i) Copy of Specification and Claims of U.S. Provisional Application No. 60/099,805
  - (ii) Copy of Specification and Claims of U.S. Provisional Application No. 60/131,965
  - (iii) Office Action dated January 24, 2006 for USSN 10/153,770 (corresponding to Ebner publication No. 2003/0092133)
5. Related Proceedings Appendix - None

Attorney/Secretary: DAS/cml

Due Date: May 5, 2006

Submitted by: David A. Steffes

Date: May 5, 2006

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/153,770	05/24/2002	Reinhard Ebner	PF470D1	1658

22195 7590 01/24/2006  
HUMAN GENOME SCIENCES INC  
INTELLECTUAL PROPERTY DEPT.  
14200 SHADY GROVE ROAD  
ROCKVILLE, MD 20850

EXAMINER

SPECTOR, LORRAINE

ART UNIT	PAPER NUMBER
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1647

DATE MAILED: 01/24/2006

Please find below and/or attached an Office communication concerning this application or proceeding.



<b>Office Action Summary</b>	Application No. 10/153,770	Applicant(s) EBNER ET AL.	
	Examiner Lorraine Spector, Ph.D.	Art Unit 1647	

**-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --**

#### Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

#### Status

- 1) ☒ Responsive to communication(s) filed on 04 November 2005.
- 2a) ☐ This action is **FINAL**.                      2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

#### Disposition of Claims

- 4) ☒ Claim(s) 29 and 50-57 is/are pending in the application.
- 4a) Of the above claim(s) 29 is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 50-57 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☒ Claim(s) 29 and 50-57 are subject to restriction and/or election requirement.

#### Application Papers

- 9) ☒ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 24 May 2002 and 27 August 2002 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

#### Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All    b) ☐ Some \* c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- \* See the attached detailed Office action for a list of the certified copies not received.

#### Attachment(s)

- |  |   |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)  | 4) <input type="checkbox"/> Interview Summary (PTO-413)<br>Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)   | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152)             |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)<br>Paper No(s)/Mail Date <u>11/4/05</u> . | 6) <input type="checkbox"/> Other: _____  |

**DETAILED ACTION*****Election/Restrictions***

Applicant's election with traverse of Invention III in the reply filed on 11/4/2005 is acknowledged. The traversal is on the ground(s) that a search of the various inventions would not be burdensome. This is not found persuasive because with respect to the antibodies, the only other invention still the subject of a pending claim, the burdensome search is shown by the different classification of the inventions, as set forth in the previous Office Action. Further, it is well known that a search of the antibody art requires not only consideration of the protein, but includes a thorough search of the antibody art as many antibodies are disclosed as having been obtained by other means, and are characterized other than by the protein to which they bind. Such a search would be an undue burden.

The requirement is still deemed proper and is therefore made FINAL.

Claims 50-57 are under consideration.

***Priority***

Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) and 120 as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosures of the prior-filed applications fail to provide adequate enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. Accordingly priority is granted only to the instant filing date, 5/24/2002.

***Information Disclosure Statement***

The information disclosure filed 11/4/2005 has been fully considered. It is noted that the relevance of references AC-BS cannot be ascertained in the absence of an alignment to the claimed sequence (or encoding DNA, as appropriate). References CC and CD have been considered but are lined-through, as they are not publications, and will not be printed on the face of the patent. Similarly, reference D and E have been lined-through, as they are not publications, and will not be printed on the face of the patent.

***Specification***

The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed.

The disclosure is objected to because of the following informalities:

It is noted that the specification refers to the protein of SEQ ID NO: 29 as Interleukin-21. However, it is noted that, while applicant may be their own lexicographer, the protein of SEQ ID NO: 29 is not the protein that has attained recognition in the art as being Interleukin-21, but rather is known in the art as IL-17C. See enclosed NCBI printout of locus AAH66262, Homo sapiens IL-21, which has a significantly different sequence than SEQ ID NO: 29, and locus AAI01836, Homo sapiens IL-17C, which is identical in sequence to SEQ ID NO: 29.

Appropriate correction is required.

***Objections and Rejections under 35 U.S.C. §§101 and 112***

35 U.S.C. 101 reads as follows:

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 50-57 are rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a specific, substantial and credible asserted utility or a well established utility.

The instant application discloses a portion of a protein which applicants designate Interleukin 21 ("IL-21") having SEQ ID NO: 29 and a nucleic acid which encodes such, SEQ ID NO: 28. The specification states at page 10 that the cDNA was isolated from an apoptotic T-cell cDNA library. At page 85 it is disclosed that the nucleic acid is expressed in skeletal muscle, fetal lung and fetal kidney, stating "very light signals of 1.8 and 3.0 kb were detected in skeletal muscle, and signals of indeterminate sizes were detected in fetal lung and fetal kidney". The significance of the nucleic acid sizes is not clear nor disclosed, nor is it clear that protein is expressed in those tissues. At page 10, it is clearly stated that SEQ ID NO: 1 is only a partial open reading frame, that is, that the complete coding sequence is not represented. At pages 12-13 there is a structural analysis of the putative protein, stating that it is related to IL-20 and IL-17 based on similarity in a number of small domains; however, there is no functional significance disclosed for those domains. The specification teaches at page 70 that probes to detect "IL-21" may be used in a variety of forensic and diagnostic methods. Other disclosed uses are in chromosome mapping, gene therapy, antisense, and tissue typing. The protein encoded thereby is stated to be useful in assays, or diagnostic or treatment methods (see page 72, for example). At page 74, it is disclosed that both "IL-21" and IL-22 modulate secretion of IL-6 from NIH-3T3 cells. There is no other specific biological activity disclosed for the protein, however, the specification contains conjecture such that the claimed may activate or inhibit proliferation, differentiation or mobilization of immune cells (page 75). At pages 76-81 a number of possible uses for the claimed protein are presented, including treatment of hyperproliferative disorders, infections disease, regeneration of tissue, chemotaxis, and binding. There is no working example in which any biological activity is demonstrated for the protein.

None of the aforementioned uses is considered to be specific, substantial and credible, as set forth in the Utility Examination Guidelines of 1/5/2001, Federal Register 66(4) beginning at page 1092. It is not predictable that "IL-21" will share function with other interleukins, nor if so, what functions would be shared. The assertion that the disclosed "IL-21" would have biological activities as set forth above cannot be accepted in the absence of supporting evidence, because

the proposed activities of the protein are merely conjectural, and the specification 'discloses' numerous mutually exclusive activities or uses, such that none can be considered to be credible without any supporting evidence. Further, the relevant literature indicates that prediction of function from structure is not accurate, and reports examples of polypeptide families wherein individual members have distinct, and sometimes even opposite, biological activities, such that the scattered similarities to other known interleukins cannot be taken to be predictive of any particular function. For example, Tischer et al. (U.S. Patent 5,194,596, cited by applicants) establishes that VEGF (a member of the PDGF, or platelet-derived growth factor, family) is mitogenic for vascular endothelial cells but not for vascular smooth muscle cells, which is opposite to the mitogenic activity of naturally occurring PDGF which is mitogenic for vascular smooth muscle cells but not for vascular endothelial cells (column 2, line 46 to column 3, line 2). The differences between PDGF and VEGF are also seen *in vivo*, wherein endothelial-pericyte associations in the eye are disrupted by intraocular administration of PDGF but accelerated by intraocular administration of VEGF (Benjamin et al., 1998, Development 125:1591-1598, cited by applicants; see Abstract and pp. 1594-1596). Generally, the art acknowledges that function cannot be predicted based solely on structural similarity to a protein found in the sequence databases. For example, Skolnick et al. (2000, Trends in Biotech. 18:34-39, cited by applicants) state that knowing the protein structure by itself is insufficient to annotate a number of functional classes, and is also insufficient for annotating the specific details of protein function (see Box 2, p. 36). Similarly, Bork (2000, Genome Research 10:398-400, cited by applicants) states that the error rate of functional annotations in the sequence database is considerable, making it even more difficult to infer correct function from a structural comparison of a new sequence with a sequence database (see especially p. 399). Such concerns are also echoed by Doerks et al. (1998, Trends in Genetics 14:248-250, cited by applicants) who state that (1) functional information is only partially annotated in the database, ignoring multi functionality, resulting in underpredictions of functionality of a new protein and (2) overpredictions of functionality occur because structural similarity often does not necessarily coincide with functional similarity. Smith et al. (1997, Nature Biotechnology 15:1222-1223, cited by applicants) remark that there are numerous cases in which proteins having very different functions share structural similarity due to evolution from a common ancestral gene. Accordingly, in view of the cited art, the skilled

artisan would not accept, without experimental confirmation, that "IL-21" would have any particular of the plethora of proposed activities.

With regard to the remaining uses asserted by applicants, no assertion of diagnostic or therapeutic use of the claimed protein can be considered to be specific or substantial, much less credible, as there is no disclosure of any condition which can be so diagnosed or treated. The disclosure that the protein may modulate immune system cell proliferation and differentiation in a dose-dependent manner is merely an invitation to experiment, and would not be considered substantial by one of skill in the art; mere homology and expression patterns is not accepted by those of skill in the art as being predictive of function, and the term "modulate" can encompass either a positive or negative effect. Finally, although there is a clear statement that the protein modulates secretion of IL-6 from NIH-3T3 cells, this does not satisfy the utility requirement because it is not clear whether this is positive or negative modulation nor under what conditions such occurs, and because it is not clear what utility such an effect would convey. In *Brenner v. Manson*, 148 U.S.P.Q. 689 (Sup. Ct., 1966), a process of producing a novel compound that was structurally analogous to other compounds which were known to possess anti-cancer activity was alleged to be useful because the compound produced thereby was potentially useful as an anti-tumor agent in the absence of evidence supporting this utility. The court expressed the opinion that all chemical compounds are "useful" to the chemical arts when this term is given its broadest interpretation. However, the court held that this broad interpretation was not the intended definition of "useful" as it appears in 35 U.S.C. § 101, which requires that an invention must have either an immediately obvious or fully disclosed "real world" utility. The instant claims are drawn protein which has undetermined function or biological significance, and polynucleotides encoding such. Until some actual and specific activity can be attributed to the protein identified in the specification as "IL-21", the claimed invention is incomplete.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to

Art Unit: 1647

make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 50-57 are also rejected under 35 U.S.C. 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific, substantial and credible asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention.

Claim 52 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The claim is directed to glycosylated protein of SEQ ID NO: 29. There are no putative glycosylation sites identified within SEQ ID NO: 29. Accordingly, there is no written description of a glycosylated form of the protein of SEQ ID NO: 29.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 51-54 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 51-53 are indefinite as there is no antecedent basis in claim 50 for a polypeptide.

Claim 54 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. Merely culturing a generic host cell will not result in production of the protein of claim 50. The claim contains no limitations that would enable the host cell to produce such a protein.

Art Unit: 1647

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 50, 54 and 55 are rejected under 35 U.S.C. 102(e) as being anticipated U.S. Patent No. 6,562,578 (Gorman et al). Gorman's SEQ ID NO: 23 is 100% identical to SEQ ID NO: 29.

Claims 50, 51, 54 and 55 are rejected under 35 U.S.C. 102(b) as being anticipated by Li et al., Reference CB cited by applicants.

Li et al. disclose IL-17C, which has an identical sequence to SEQ ID NO: 29, and Fusion proteins comprising such. As the protein was in solution, it meets the limitations of claim 25. Li further discloses that IL-17C induces production of TNF-alpha and IL-1 beta.

### ***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later



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invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claim 53 is rejected under 35 U.S.C. 103(a) as being unpatentable over Li et al. in view of Mikayama et al., U.S. Patent No. 5,264,209.

Claim 53 includes the limitation that the protein is conjugated to polyethylene glycol (PEG).

The teachings of Li et al. are discussed above. Li does not disclose conjugation to PEG.

Mikayama et al. disclose modification of human IL-6 proteins with PEG. The stated advantages are extended plasma half-life (abstract), which is well known in the art as being an advantage to attaching PEG to a protein.

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time the invention was made to derivitize IL-17C as taught by Li by attaching PEG as taught by Mikayama for the purpose of extending serum half-life. The person of ordinary skill in the art would be motivated to do so in view of Li's teaching of the biological activity of IL-17C, namely induction of TNF-alpha and IL-1 beta.

Claims 56 and 57 are rejected under 35 U.S.C. 103(a) as being unpatentable over Li et al. in view of Chen et al., U.S. Patent No. 6,569,645.

Claims 56 and 57 are drawn to fragments of the protein of SEQ ID NO: 29.

The teachings of Li et al. are discussed above. Li does not disclose fragments of the protein.

Chen et al. disclose proteins with homology to IL-17. They also fragments of the proteins. For example, at paragraph DETX (150) they state:

Detailed Description Text - DETX (213):

Screening assays for drug candidates are designed to identify compounds that bind to or complex with the polypeptides encoded by the genes identified herein or a biologically active fragment thereof, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins.

Art Unit: 1647

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time the invention was made to synthesize a biologically active fragment of IL-17C to be used in screening assays as disclosed by Chen et al.

### *Conclusion*

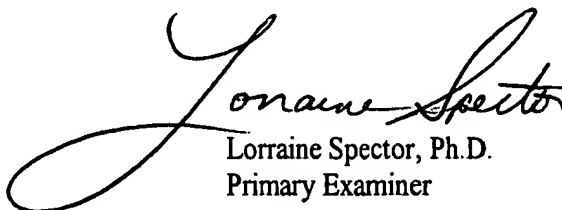
Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Lorraine M. Spector. Dr. Spector can normally be reached Monday through Friday, 9:00 A.M. to 3:00 P.M. at telephone number 571-272-0893.

If attempts to reach the Examiner by telephone are unsuccessful, please contact the Examiner's supervisor, Ms. Brenda Brumback, at telephone number 571-272-0961.

Certain papers related to this application may be submitted to Technology Center 1600 by facsimile transmission. The faxing of such papers must conform with the notices published in the Official Gazette, 1156 OG 61 (November 16, 1993) and 1157 OG 94 (December 28, 1993) (see 37 C.F.R. § 1.6(d)). NOTE: If Applicant does submit a paper by fax, the original signed copy should be retained by applicant or applicant's representative. NO DUPLICATE COPIES SHOULD BE SUBMITTED so as to avoid the processing of duplicate papers in the Office.

Official papers filed by fax should be directed to **571-273-8300**. Faxed draft or informal communications with the examiner should be directed to **571-273-0893**.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

  
Lorraine Spector, Ph.D.  
Primary Examiner

In re Chen, et al.  
Application No. 09/854,208  
Appeal Brief

**10. RELATED PROCEEDINGS APPENDIX**

None

U.S. PATENT AND TRADEMARK OFFICE  
PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Docket No.	<b>PF470PP2</b>	Type a plus sign (+) inside this box →	+
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♦ INVENTOR (S) / APPLICANT (S)			
Last Name	First Name	Middle Initial	Residence (City and Either State or Foreign Country)
EBNER	Reinhard		Gaithersburg, MD
RUBEN	Steven	M.	Olney, MD

♦ TITLE OF THE INVENTION (280 characters max)			
Interleukins-21 and 22			
♦ CORRESPONDENCE ADDRESS			
Robert H. Benson (Reg. No. 30,446), A. Anders Brookes (Reg. No. 36,373), James H. Davis (Reg. No. 40,582) and Kenley K. Hoover (Reg. No. 40,302) of Human Genome Sciences, Inc. 9410 Key West Avenue, Rockville, Maryland, 20850.		Ph: 301-309-8504 Fax: 301-309-8439	
State	Maryland	Zip Code	20850 Country U.S.

♦ ENCLOSED APPLICATION PARTS (check all that apply)			
<input checked="" type="checkbox"/> Specification	No. of Pages Pages: 1-102 102	<input checked="" type="checkbox"/> Claims	No. of pages: 9 Pages: 103-111
<input checked="" type="checkbox"/> Abstract	No. of Pages Page: 112	<input checked="" type="checkbox"/> Sequence Listing	No. of pages: 1-20 Pages: 20
		<input checked="" type="checkbox"/> Figures	No. of sheets: 10 containing Figs. 1-7
♦ METHOD OF PAYMENT (check one)			
<input type="checkbox"/> A check or money order is enclosed to cover the Provisional filing fee		PROVISIONAL	
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number 08-3425		FILING FEE AMOUNT (\$)	\$150.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE



Date

9/10/98

TYPE OR PRINTED NAME

A. Anders Brookes

REGISTRATION No.  
(If appropriate)

(Reg. No. 36,373)

☐ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

AAB/JK/lcc

## Interleukins-21 and 22

### *Field of the Invention*

The present invention relates to two novel human genes, each of which encodes a polypeptide which is a member of the Interleukin family. More specifically, the present invention relates to a polynucleotide encoding a novel human polypeptide named Interleukin-21, or "IL-21". The present invention also relates to a polynucleotide encoding a novel human polypeptide named Interleukin-22, or "IL-22". This invention also relates to IL-21 and IL-22 polypeptides, as well as vectors, host cells, antibodies directed to IL-21 and IL-22 polypeptides, and recombinant methods for producing the same. Also provided are diagnostic methods for detecting disorders related to the immune system, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of IL-21 and IL-22 activity.

### *Background of the Invention*

Cytokines typically exert their respective biochemical and physiological effects by binding to specific receptor molecules. Receptor binding then stimulates specific signal transduction pathways (Kishimoto, T., *et al.*, *Cell* **76**:253-262 (1994)). The specific interactions of cytokines with their receptors are often the primary regulators of a wide variety of cellular processes including activation, proliferation, and differentiation (Arai, K. -I, *et al.*, *Ann. Rev. Biochem.* **59**:783-836 (1990); Paul, W. E. and Seder, R. A., *Cell* **76**:241-251 (1994)).

Human interleukin (IL)-17, a closely related homolog of the molecules of the present invention, was only recently identified. IL-17 is a 155 amino acid polypeptide which was molecularly cloned from a CD4+ T-cell cDNA library (Yao, Z., *et al.*, *J. Immunol.* **155**:5483-5486 (1995)). The IL-17 polypeptide contains an N-terminal signal peptide and contains approximately 72% identity at the amino acid level with a T-cell trophic herpesvirus saimiri (HVS) gene designated HVS13. High levels of IL-17 are secreted from CD4-positive primary peripheral blood leukocytes (PBL) upon stimulation (Yao, Z., *et al.*, *Immunity* **3**:811-821 (1995)). Treatment of fibroblasts with IL-17, HVS13, or another murine homologue, designated CTLA8, activate signal transduction pathways and result in the stimulation of the NF- $\kappa$ B transcription factor family, the secretion of IL-6, and the costimulation of T-cell proliferation (Yao, Z., *et al.*, *Immunity* **3**:811-821 (1995)).

An HVS13-Fc fusion protein was used to isolate a murine IL-17 receptor molecule which does not appear to belong to any of the previously described cytokine receptor families (Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). The murine IL-17 receptor (mIL-17R) is predicted to encode a type I transmembrane protein of 864 amino acids with an apparent molecular mass of 97.8 kDa. mIL-17R is predicted to possess an N-terminal signal peptide with a cleavage site between alanine-31 and serine-32. The molecule also contains a 291 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 521 amino acid cytoplasmic tail. A soluble recombinant IL-17R molecule consisting of 323 amino acids of the extracellular domain of IL-17R fused to the Fc portion of human immunoglobulin IgG1 was able to significantly inhibit IL-17-induced IL-6 production by murine NIH-3T3 cells (*supra*).

Interestingly, the expression of the IL-17 gene is highly restricted. It is typically observed primarily in activated T-lymphocyte memory cells (Broxmeyer, H. J. *Exp. Med.* 183:2411-2415 (1996); Fossiez, F., *et al.*, *J. Exp. Med.* 183:2593-2603 (1996)). Conversely, the IL-17 receptor appears to be expressed in a large number of cells and tissues (Rouvier, E., *et al.*, *J. Immunol.* 150:5445-5456 (1993); Yao, Z., *et al.*, *J. Immunol.* 155:5483-5486 (1995)). It remains to be seen, however, if IL-17 itself can play an autocrine role in the expression of IL-17. IL-17 has been implicated as a causative agent in the expression of IL-6, IL-8, G-CSF, Prostaglandin E (PGE<sub>2</sub>), and intracellular adhesion molecule (ICAM)-1 (Fossiez, F., *supra*; Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). Each of these molecules possesses highly relevant and potentially therapeutically valuable properties. For instance, IL-6 is involved in the regulation of hematopoietic stem and progenitor cell growth and expansion (Ikebuchi, K., *et al.*, *Proc. Natl. Acad. Sci. USA* 84:9035-9039 (1987); Gentile, P. and Broxmeyer, H. E. *Ann. N.Y. Acad. Sci. USA* 628:74-83 (1991)). IL-8 exhibits a myelosuppressive activity for stem cells and immature subsets of myeloid progenitors (Broxmeyer, H. E., *et al.*, *Ann. Hematol.* 71:235-246 (1995); Daly, T. J., *et al.*, *J. Biol. Chem.* 270:23282-23292 (1995)). G-CSF acts both early and late to activate and stimulate hematopoiesis in general, and more specifically on neutrophil hematopoiesis, while PGE<sub>2</sub> enhances erythropoiesis, suppresses lymphopoiesis and myelopoiesis in general, and strongly suppresses monocytopenia (Broxmeyer, H. E. *Amer. J. Ped. Hematol./Oncol.* 14:22-30 (1992); Broxmeyer, H. E. and Williams, D. E. *CRC Crit. Rev. Oncol./Hematol.* 8:173-226 (1988)).

Thus, there is a need for polypeptides that function as immunoregulatory molecules and, thereby, modulate the transfer of an extracellular signal ultimately to the nucleus of the cell, since disturbances of such regulation may be involved in disorders relating to cellular activation, hemostasis, angiogenesis, tumor metastasis, cellular

migration and ovulation, as well as neurogenesis. Therefore, there is a need for identification and characterization of such human polypeptides which can play a role in detecting, preventing, ameliorating or correcting such disorders.

### ***Summary of the Invention***

5       The present invention relates to novel polynucleotides and the encoded polypeptides of IL-21 and IL-22. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting disorders related to the polypeptides, and therapeutic methods for treating such disorders. The invention  
10       further relates to screening methods for identifying binding partners of IL-21 and IL-22.

### ***Brief Description of the Drawings***

15       Figure 1 shows the partial nucleotide sequence (SEQ ID NO:1) and the deduced amino acid sequence (SEQ ID NO:2) of IL-21. The locations of conserved Domains I-IV (see below) are underlined and labeled as such.

20       Figures 2A and 2B show the nucleotide sequence (SEQ ID NO:3) and the deduced amino acid sequence (SEQ ID NO:4) of IL-22. The locations of conserved Domains I-IV (see below) are underlined and labeled as such. The locations of two potential N-linked glycosylation sites are identified by a bolded asparagine symbol (N) accompanied by a bolded pound sign (#) located above the initial nucleotide of the codon encoding the corresponding asparagine.

25       Figures 3A and 3B show the regions of identity between the amino acid sequence of a partial-length IL-21 protein, and the IL-22 protein (designated IL-21.aa and IL-22.aa in the figure), the full-length IL-21 protein (designated IL-21FL.aa in the figure) the amino acid sequence of IL-20 (designated IL20.aa in the figure and disclosed in copending U.S. Provisional Application Serial No. 60/060,140; filed September 26, 1997; SEQ ID NO:8), and human Interleukin-17 (designated IL-17.aa in the figure; GenBank Accession No. U32659; SEQ ID NO:5), mouse Interleukin-17  
30       (designated mIL-17.aa in the figure; GenBank Accession No. U43088; SEQ ID NO:6), and viral Interleukin-17 (designated vIL-17.aa in the figure; GenBank Accession No. X64346; SEQ ID NO:7), as determined by the MegAlign component of the computer program DNA\*Star (DNASTAR, Inc.) using the default parameters.

35       Figure 4 shows an analysis of the partial IL-21 amino acid sequence (SEQ ID NO:2). Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity;

amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index" or "Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the IL-21 protein, that is, regions from which epitope-bearing peptides of the invention can be determined. Polypeptides and polynucleotides encoding polypeptides comprising the domains defined by these graphs are contemplated by the present invention.

Figure 5 shows an analysis of the IL-22 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index" or "Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the IL-22 protein, that is, regions from which epitope-bearing peptides of the invention can be determined. Polypeptides and polynucleotides encoding polypeptides comprising the domains defined by these graphs are contemplated by the present invention.

The data presented in Figure 5 are also represented in tabular form in Table II. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIII. The column headings refer to the following features of the amino acid sequence presented in Figure 5 and Table II: "Res": amino acid residue of SEQ ID NO:4 or Figures 2A and 2B; "Position": position of the corresponding residue within SEQ ID NO:4 or Figures 2A and 2B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Alpha, Amphipathic Regions - Eisenberg; X: Beta, Amphipathic Regions - Eisenberg; XI: Flexible Regions - Karplus-Schulz; XII: Antigenic Index - Jameson-Wolf; and XIII: Surface Probability Plot - Emini.

Figures 6A and 6B show the nucleotide sequence (SEQ ID NO:28) and the deduced amino acid sequence (SEQ ID NO:29) of the full-length IL-21. The locations of conserved Domains I-IV (identical to those shown in Figure 1) and of conserved Domains V-VII are underlined and labeled as such. A predicted signal peptide from methionine-1 to alanine-18 is double underlined.

Figure 7 shows an analysis of a full-length IL-21 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index" or "Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of a full-length IL-21 protein, that is, regions from which epitope-bearing peptides of the invention can be determined. Polypeptides and



polynucleotides encoding polypeptides comprising the domains defined by these graphs are contemplated by the present invention.

The data presented in Figure 7 are also represented in tabular form in Table I. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 7 and Table I: "Res": amino acid residue of SEQ ID NO:29 or Figures 6A and 6B; "Position": position of the corresponding residue within SEQ ID NO:29 or Figures 6A and 6B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

## *Detailed Description*

### **Definitions**

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. However, a nucleic acid contained in a clone that is a member of a library (e.g., a genomic or cDNA library) that has not been isolated from other members of the library (e.g., in the form of a homogeneous solution containing the clone and other members of the library) or which is contained on a chromosome preparation (e.g., a chromosome spread), is not "isolated" for the purposes of this invention.

In the present invention, a "secreted" IL-21 or IL-22 protein refers to a protein capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as an IL-21 or IL-22 protein released into the extracellular space without necessarily containing a signal sequence. If the IL-21 or IL-22 secreted protein is released into the extracellular space, the IL-21 or IL-22 secreted protein can undergo extracellular processing to produce a "mature" IL-21 or

IL-22 protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

As used herein, an IL-21 or IL-22 "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:1 or in SEQ ID NO:3, respectively, or the cDNA contained within the respective clones deposited with the ATCC. For example, the IL-21 or IL-22 polynucleotide can contain the nucleotide sequence of the full-length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, an IL-21 or IL-22 "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

A representative clone containing all or most of the sequence for SEQ ID NO:1 (designated HTGED19) was deposited with the American Type Culture Collection ("ATCC") on March 5, 1998, and was given the ATCC Deposit Number 209666. In addition, a representative clone containing all or most of the sequence for SEQ ID NO:3 (designated HFPBX96) was also deposited with the ATCC on March 5, 1998, and was given the ATCC Deposit Number 209665. The ATCC is located at 10801 University Blvd., Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

An IL-21 "polynucleotide" also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:1, the complement thereof, or the cDNA within the deposited clone. Further, An IL-22 "polynucleotide" also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:3, the complement thereof, or the cDNA within the deposited clone. "Stringent hybridization conditions" refers to an overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

Also contemplated are nucleic acid molecules that hybridize to the IL-21 and the IL-22 polynucleotides at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE

5 (20X SSPE = 3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50°C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

10 Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

15 Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a polyA+ stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

20 The IL-21 and IL-22 polynucleotides can be composed of any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, the IL-21 and IL-22 polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the IL-21 polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. IL-21 polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

35 IL-21 and IL-22 polypeptides can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The IL-21 and IL-22 polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art.

"SEQ ID NO:1" refers to an IL-21 polynucleotide sequence while "SEQ ID NO:2" refers to an IL-21 polypeptide sequence. Likewise, "SEQ ID NO:3" refers to an IL-22 polynucleotide sequence while "SEQ ID NO:4" refers to an IL-22 polypeptide sequence.

30 An IL-21 polypeptide "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of an IL-21 polypeptide, including mature forms, as measured in a particular biological assay, with or without dose-dependency. In addition, an IL-22 polypeptide "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical  
35 to, an activity of an IL-22 polypeptide, including mature forms, as measured in a particular biological assay, with or without dose-dependency. In the case where dose-dependency does exist, it need not be identical to that of the IL-21 or IL-22

polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the IL-21 or IL-22 polypeptides (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the IL-21 polypeptide).

### **IL-21 and IL-22 Polynucleotides and Polypeptides**

Clone HTGED19, encoding IL-21, was isolated from a cDNA library derived from apoptotic T-cells. This clone contains the entire coding region identified as SEQ ID NO:2. The deposited clone contains a cDNA having a total of 705 nucleotides, which encodes a partial predicted open reading frame of 87 amino acid residues (see Figure 1). The partial open reading frame begins at a point in the complete IL-21 ORF such that the "G" in position 1 of SEQ ID NO:1 is actually in position 3 of a coding triplet. As such, the partial predicted IL-21 polypeptide sequence is shown beginning in-frame with an alanine residue at position 1 of SEQ ID NO:2. The alanine residue at position 1 of SEQ ID NO:2 is encoded by nucleotides 2-4 of the nucleotide sequence shown as SEQ ID NO:1. The ORF shown as SEQ ID NO:2 ends at a stop codon at nucleotide position 263-265 of the nucleotide sequence shown as SEQ ID NO:1. The predicted molecular weight of the partial IL-21 protein should be about 9,558 Daltons.

An initial BLAST analysis of the expression of the IL-21 cDNA sequence against the HGS EST database has also revealed a highly specific expression of this cDNA clone. In such an analysis, the HTGED19 cDNA sequence appears to be found only in apoptotic T-cells. Thus, IL-21 appears to be expressed in a highly restricted pattern limited to apoptotic T-cells, and, for example, other subpopulations of lymphocytes or other cells in a state of activation or quiescence.

Clone HTGED19, encoding IL-21, was used to screen a panel of bacterial artificial chromosomes containing various segments of human genomic DNA (Research Genetics, Inc.). A positive clone was sequenced to identify potential splice donor and acceptor sites. Analysis of several sites revealed an upstream partial ORF that, when placed immediately 5' and in frame with the existing IL-21 DNA sequence, generated a complete ORF which encodes a polypeptide with additional sequence identity to the IL-17 family (See Figures 3A and 3B). A full-length IL-21 clone has been constructed from the HTGED19 cDNA clone and the upstream genetic material. The nucleotide sequence of the full-length IL-21 clone contains the entire coding region identified as SEQ ID NO:29. The resultant clone contains an insert having a total of 1067 nucleotides, which encodes a predicted open reading frame of 197 amino acid residues (see Figures 6A and 6B). The open reading frame begins at nucleotide position 34 in

the complete IL-21 polynucleotide shown as SEQ ID NO:28 (Figures 6A and 6B). The ORF ends at a stop codon at nucleotide position 625-627 of the nucleotide sequence shown as SEQ ID NO:28 (Figures 6A and 6B). The predicted molecular weight of the IL-21 polypeptide shown in Figures 6A and 6B and as SEQ ID NO:29 should be about 21,764 Daltons.

Further BLAST analysis of the expression of the full-length IL-21 cDNA sequence against the HGS EST database has also revealed a highly specific expression of this cDNA clone. In such an analysis, the full-length HTGED19 cDNA sequence appears to be found only in apoptotic T-cells. Thus, IL-21 appears to be expressed in a highly restricted pattern limited to apoptotic T-cells, and, for example, other subpopulations of lymphocytes or other cells in a state of activation or quiescence.

Clone HFPBX96, encoding IL-22, was isolated from a cDNA library derived from epileptic frontal cortex. This clone contains the entire coding region identified as SEQ ID NO:4. The deposited clone contains a cDNA having a total of 1,642 nucleotides, which encodes a partial predicted open reading frame of 160 amino acid residues (see Figure 2). The partial open reading frame begins at a point in the complete IL-22 ORF such that the "G" in position 1 of SEQ ID NO:3 is actually in position two of a coding triplet. As such, the partial predicted IL-22 polypeptide sequence is shown beginning in-frame with an asparagine residue at position 1 of SEQ ID NO:4. The asparagine residue at position 1 of SEQ ID NO:4 is encoded by nucleotides 3-5 of the nucleotide sequence shown as SEQ ID NO:3. The ORF shown as SEQ ID NO:4 ends at a stop codon at nucleotide position 483-485 of the nucleotide sequence shown as SEQ ID NO:3. The predicted molecular weight of the partial IL-22 protein should be about 17,436 Daltons.

Using BLAST and MegAlign analyses, SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:29 were each found to be highly homologous to several members of the Interleukin family. Particularly, SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:29 contain at least four domains homologous to the translation products of the human mRNA for Interleukin (IL)-20 (copending U.S. Provisional Application Serial No. 60/060,140; filed September 26, 1997; SEQ ID NO:8), IL-17 (GenBank Accession No. U32659; SEQ ID NO:5; see also Figures 3A and 3B), the murine mRNA for Interleukin (IL)-17 (GenBank Accession No. U43088; SEQ ID NO:6; see also Figures 3A and 3B), and the human viral mRNA for Interleukin (IL)-17 (GenBank Accession No. X64346; SEQ ID NO:7; see also Figures 3A and 3B). Specifically, the molecules of the present invention, in particular, SEQ ID NO:2, SEQ ID NO:4 SEQ ID NO:29, share a high degree of sequence identity with IL-20, IL-17, mIL-17, and vIL-17 in the following conserved domains: (a) a predicted NXDPXRY domain (where X

represents any amino acid) located at about amino acids valine-3 to proline-11 of SEQ ID NO:2, serine-57 to proline-64 of SEQ ID NO:4, valine-113 to proline-121 of SEQ ID NO:29, and asparagine-79 to proline-86 of the human IL-17 amino acid sequence (SEQ ID NO:5); (b) a predicted CLCXGC domain (where X represents any amino acid) located at about amino acids cysteine-19 to cysteine-24 of SEQ ID NO:2, cysteine-72 to cysteine-77 of SEQ ID NO:4, cysteine-129 to cysteine-134 of SEQ ID NO:29, and cysteine-94 to cysteine-99 of the human IL-17 amino acid sequence (SEQ ID NO:5); (c) a predicted LVLRRXP domain (where X represents any amino acid) located at about amino acids leucine-46 to proline-52 of SEQ ID NO:2, valine-99 to proline-105 of SEQ ID NO:4, leucine-156 to proline-162 of SEQ ID NO:29, and leucine-120 to proline-126 of the human IL-17 amino acid sequence (SEQ ID NO:5); and (d) a predicted VXVGCTCV domain (where X represents any amino acid) located at about amino acids valine-75 to valine-82 of SEQ ID NO:2, isoleucine-121 to valine-128 of SEQ ID NO:4, valine-187 to valine-192 of SEQ ID NO:29, and valine-140 to valine-147 of the human IL-17 amino acid sequence (SEQ ID NO:5). In addition, the full-length IL-21 molecule shown in Figures 6A and 6B (SEQ ID NO:29) exhibits several additional conserved domains when compared with IL-20 and the other members of the IL-17 family as shown in Figures 3A and 3B). These conserved Domains are underlined in Figures 6A and 6B and are labeled as conserved Domains V, VI, and VII. Specifically, the molecules of the present invention, in particular, SEQ ID NO:29, share a high degree of sequence identity with IL-20, IL-17, mIL-17, and vIL-17 in the following conserved domains: (a) a predicted PXCXSAE domain (where X represents any amino acid) located at about amino acids proline-34 to glutamic acid-40 of SEQ ID NO:29; (b) a predicted PXXLVS domain (where X represents any amino acid) located at about amino acids proline-63 to serine-68 of SEQ ID NO:29; and (c) a predicted RSXSPW domain (where X represents any amino acid) located at about amino acids arginine-104 to tryptophan-109 of SEQ ID NO:29. These polypeptide fragments of IL-21 and IL-22 are specifically contemplated in the present invention. Because each of these IL-17 and IL-17-like molecules is thought to be important immunoregulatory molecules, the homology between these IL-17 and IL-17-like molecules and IL-21 and IL-22 suggests that IL-21 and IL-22 may also be important immunoregulatory molecules.

Moreover, based on their apparent sequence identities with IL-17 and IL-20 (see Figures 3A and 3B), the full-length IL-21 and IL-22 polypeptides are each likely to have an amino terminal secretory signal peptide leader sequence. Since the present invention appears to be partial cDNA clones of the IL-21 (SEQ ID NOs:1 and 2) and IL-22 (SEQ ID NOs:3 and 4) molecules (in addition to the full-length IL-21 molecule

shown as SEQ ID NOs:28 and 29), it is also contemplated that the translation products of SEQ ID NOs:2 and 4 of the present invention will be caused to enter the cellular secretory pathway by virtue of being expressed as a fusion proteins comprising several different portions of the N-terminus of the IL-20 molecule of copending U.S. Provisional Application Serial No. 60/060,140 fused to the known coding sequence of the IL-21 or IL-22 molecules of the present invention. Such expression constructs will secrete hybrid IL-20/IL-21 or IL-20/IL-22 molecules from the host cell. The mature IL-21 protein used in these fusion proteins encompasses about amino acids 1-145, while the IL-20/21 fusion protein encompasses about the 104 or 113 N-terminal amino acids of IL-20 encoded in frame with about amino acids 1-145 of the IL-21 of SEQ ID NO:2. These polypeptide fragments of IL-21 are specifically contemplated in the present invention. In addition, the mature IL-22 protein used to generate these fusion proteins encompasses about amino acids 1-160, while the IL-20/22 fusion protein encompasses about the 95, 104 or 113 N-terminal amino acids of IL-20 encoded in frame with about amino acids 1-160 of the IL-22 of SEQ ID NO:4. These polypeptide fragments of IL-22 are specifically contemplated in the present invention.

The IL-21 and IL-22 nucleotide sequences identified as SEQ ID NO:1 and SEQ ID NO:3, respectively, were assembled from partially homologous ("overlapping") sequences obtained from the deposited clones. The IL-21 nucleotide sequence identified as SEQ ID NO:28 was assembled from partially homologous ("overlapping") sequences obtained from the deposited clone and a genomic DNA clone. The overlapping sequences specific to the partial IL-21 and IL-22 molecules of the invention and the full-length IL-21 molecule of the invention were each assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in three final sequences identified as SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:28.

Therefore, SEQ ID NO:1 and the translated SEQ ID NO:2; SEQ ID NO:3 and the translated SEQ ID NO:4; and SEQ ID NO:28 and the translated SEQ ID NO:29, are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:28 are useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:28, or the cDNA contained in the respective deposited cDNA clones. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:2 and SEQ ID NO:29 may be used to generate antibodies which bind



specifically to IL-21 and polypeptides identified from SEQ ID NO:4 may be used to generate antibodies which bind specifically to IL-22.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:1 and the predicted translated amino acid sequence identified as SEQ ID NO:2, and the generated nucleotide sequence identified as SEQ ID NO:28 and the predicted translated amino acid sequence identified as SEQ ID NO:29, but also a sample of plasmid DNA containing a human cDNA of IL-21 deposited with the ATCC. In addition, the present invention also provides not only the generated nucleotide sequence identified as SEQ ID NO:3 and the predicted translated amino acid sequence identified as SEQ ID NO:4, but also a sample of plasmid DNA containing a human cDNA of IL-22 deposited with the ATCC. Accordingly, the nucleotide sequence of the deposited IL-21 and IL-22 clones can be readily determined by sequencing the deposited clone in accordance with known methods. The predicted IL-21 and IL-22 amino acid sequences can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by the deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human IL-21 or IL-22 cDNAs, collecting the protein, and determining its sequence.

The present invention also relates to the IL-21 gene corresponding to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:28, SEQ ID NO:29 or the deposited clone which encodes a partial IL-21. The present invention further relates to the IL-22 gene corresponding to SEQ ID NO:3, SEQ ID NO:4, or the deposited clone which encodes IL-22. The IL-21 and IL-22 genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequences and identifying or amplifying the IL-21 and IL-22 genes from appropriate sources of genomic material.

Also provided in the present invention are species homologs of IL-21 and IL-22. Species homologs may be isolated and identified by making suitable probes or

primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homolog.

The IL-21 and IL-22 polypeptides can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The IL-21 and IL-22 polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein. It is often advantageous to include an additional amino acids which comprise secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

IL-21 and IL-22 polypeptides are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of an IL-21 or IL-22 polypeptide, including the secreted polypeptide, can be substantially purified by the one-step method described in the publication by Smith and Johnson (*Gene* 67:31-40 (1988)). IL-21 and IL-22 polypeptides also can be purified from natural or recombinant sources using antibodies of the invention raised against the IL-21 and IL-22 proteins, respectively, in methods which are well known in the art.

#### **Polynucleotide and Polypeptide Variants**

"Variant" refers to a polynucleotide or polypeptide differing from the IL-21 and IL-22 polynucleotides or polypeptides, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the IL-21 and IL-22 polynucleotide or polypeptide.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the IL-21 or IL-22 polypeptides. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be inserted, deleted or substituted with another nucleotide. The query sequence may be an entire sequence shown of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, the ORF (open reading frame) of either IL-21 or IL-22, or any fragement specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to (or 10%, 5%, 4%, 3%, 2% or 1% different from) a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag and colleagues (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting (uridine residues (U) to thymidine residues (T)). The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, but not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB algorithm does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence ((number of bases at the 5' and 3' ends not matched)/(total number of bases in the query sequence)), so 10% is subtracted from the percent identity score calculated by the

FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence which is, at least, for example, 95% "identical" to (or 5% different from) a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (insertions and deletions are collectively referred to as "indels" in the art) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino- or carboxy-terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to (or 10%, 5%, 4%, 3%, 2% or 1% different from), for instance, the amino acid sequences shown in SEQ ID NO:2, or that shown in SEQ ID NO:4, or to the amino acid sequence encoded by deposited cDNA clones, can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag and colleagues (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity.

- 5 For subject sequences truncated at the N- and C-termini, relative to the the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of
- 10 the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are
- 15 considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

- For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the
- 20 N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence), so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues
- 25 were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue
- 30 positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

- The IL-21 and IL-22 variants may contain alterations in the coding regions,
- 35 non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants

produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. IL-21 and IL-22 polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring IL-21 and IL-22 variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (*Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the IL-21 and IL-22 polypeptides. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. Ron and coworkers reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues (*J. Biol. Chem.* **268**:2984-2988 (1993)). Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein (Dobeli, *et al.*, *J. Biotechnol.* **7**:199-216 (1988)).

In the present case, since the IL-21 and IL-22 proteins of the invention are highly related to the Interleukin-17-like polypeptide family, deletions of N-terminal amino acids up to the cysteine at position 19 of SEQ ID NO:2 and up to the cysteine at position 29 of SEQ ID NO:4 may retain some biological activity. Polypeptides having further N-terminal deletions including the cysteine-19 residue in SEQ ID NO:2 and the cysteine-29 residue in SEQ ID NO:4 would not be expected to retain such biological activities because it is likely that these residues are required for forming a disulfide bridge to provide structural stability which is needed for receptor binding and signal transduction.

However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 or IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 or IL-22 proteins are removed from the N-termini of the respective proteins. Whether a particular polypeptide lacking

N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:2, up to the cysteine residue at position number 19, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the cysteine residue at position number 29, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues  $n^1$ -87 of SEQ ID NO:2, where  $n^1$  is an integer in the range of 1 to 18, and 19 is the position of the first residue from the N-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for the receptor binding activity of the IL-21 protein. Likewise, the present invention provides polypeptides comprising the amino acid sequence of residues  $n^2$ -160 of SEQ ID NO:4, where  $n^2$  is an integer in the range of 1 to 28, and 29 is the position of the first residue from the N-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for the receptor binding activity of the IL-22 protein.

More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-87, 2-87, 3-87, 4-87, 5-87, 6-87, 7-87, 8-87, 9-87, 10-87, 11-87, 12-87, 13-87, 14-87, 15-87, 16-87, 17-87, 18-87, and 19-87 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided. The invention also provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-160, 2-160, 3-160, 4-160, 5-160, 6-160, 7-160, 8-160, 9-160, 10-160, 11-160, 12-160, 13-160, 14-160, 15-160, 16-160, 17-160, 18-160, 19-160, 20-160, 21-160, 22-160, 23-160, 24-160, 25-160, 26-160, 27-160, 28-160, and 29-160 of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

In addition, since the IL-21 and IL-22 proteins of the invention are highly related to the IL-17-like polypeptide family, deletions of C-terminal amino acids up to the leucine at position 83 of SEQ ID NO:2 and up to the proline at position 129 of SEQ ID NO:4 may retain some biological activity. Polypeptides having further C-terminal deletions including the leucine residue at position 83 of SEQ ID NO:2 and the proline at position 129 of SEQ ID NO:4 would not be expected to retain such biological activities since these residues are in the beginning of the conserved domain required for biological activities.

However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 and IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 and IL-22 proteins are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:2, up to the leucine residue at position 83 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the proline residues at position 129 of SEQ ID NO:4. In particular, the present invention provides polypeptides having the amino acid sequence of residues 1-m<sup>1</sup> of the amino acid sequence in SEQ ID NO:2, where m<sup>1</sup> is any integer in the range of 83 to 87, and residue 82 is the position of the first residue from the C-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for activity of the IL-21 protein. In addition, the present invention also provides polypeptides having the amino acid sequence of residues 1-m<sup>2</sup> of the amino acid sequence in SEQ ID NO:4, where m<sup>2</sup> is any integer in the range of 129 to 160, and residue 128 is the position of the first residue from the C-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for activity of the IL-22 protein.

More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-83, 1-84, 1-85, 1-86, and 1-87 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided. The present invention also provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-129, 1-130, 1-131, 1-132, 1-133, 1-134, 1-135, 1-136, 1-137, 1-138, 1-139, 1-140, 1-141, 1-142, 1-143, 1-144, 1-145, 1-146, 1-147, 1-148, 1-149, 1-150, 1-151, 1-152, 1-153, 1-154, 1-155, 1-156, 1-157, 1-158, 1-159, and 1-160 of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-21, which may be described



generally as having residues  $n^1$ - $m^1$  of SEQ ID NO:2, where  $n^1$  and  $m^1$  are integers as described above. Likewise, the invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-22, which may be described generally as having residues  $n^2$ - $m^2$  of SEQ ID NO:4, where  $n^2$  and  $m^2$  are integers as described above.

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers conducted extensive mutational analysis of human cytokine IL-1a (*J. Biol. Chem.* 268:22105-22111 (1993)). They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]" (see, Abstract). In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

As mentioned above, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 or IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 or IL-22 proteins are removed from the N-termini of the respective proteins. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-21

polypeptide shown in SEQ ID NO:2, up to the valine residue at position number 82, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the aspartic acid residue at position number 155, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues  $n^3$ -87 of SEQ ID NO:2, where  $n^3$  is an integer in the range of 1 to 82, and 83 is the position of the first residue from the N-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for immunogenic activity of the IL-21 protein. Likewise, the present invention provides polypeptides comprising the amino acid sequence of residues  $n^4$ -160 of SEQ ID NO:4, where  $n^4$  is an integer in the range of 1 to 155, and 156 is the position of the first residue from the N-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for immunogenic activity of the IL-22 protein.

More in particular, the invention provides polynucleotides encoding polypeptides comprising the amino acid sequence of residues R-2 to V-87; V-3 to V-87; D-4 to V-87; T-5 to V-87; D-6 to V-87; E-7 to V-87; D-8 to V-87; R-9 to V-87; Y-10 to V-87; P-11 to V-87; Q-12 to V-87; K-13 to V-87; L-14 to V-87; A-15 to V-87; F-16 to V-87; A-17 to V-87; E-18 to V-87; C-19 to V-87; L-20 to V-87; C-21 to V-87; R-22 to V-87; G-23 to V-87; C-24 to V-87; I-25 to V-87; D-26 to V-87; A-27 to V-87; R-28 to V-87; T-29 to V-87; G-30 to V-87; R-31 to V-87; E-32 to V-87; T-33 to V-87; A-34 to V-87; A-35 to V-87; L-36 to V-87; N-37 to V-87; S-38 to V-87; V-39 to V-87; R-40 to V-87; L-41 to V-87; L-42 to V-87; Q-43 to V-87; S-44 to V-87; L-45 to V-87; L-46 to V-87; V-47 to V-87; L-48 to V-87; R-49 to V-87; R-50 to V-87; R-51 to V-87; P-52 to V-87; C-53 to V-87; S-54 to V-87; R-55 to V-87; D-56 to V-87; G-57 to V-87; S-58 to V-87; G-59 to V-87; L-60 to V-87; P-61 to V-87; T-62 to V-87; P-63 to V-87; G-64 to V-87; A-65 to V-87; F-66 to V-87; A-67 to V-87; F-68 to V-87; H-69 to V-87; T-70 to V-87; E-71 to V-87; F-72 to V-87; I-73 to V-87; H-74 to V-87; V-75 to V-87; P-76 to V-87; V-77 to V-87; G-78 to V-87; C-79 to V-87; T-80 to V-87; C-81 to V-87; and V-82 to V-87 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

Further, the invention provides polynucleotides encoding polypeptides comprising the amino acid sequence of residues S-2 to P-160; A-3 to P-160; R-4 to P-160; A-5 to P-160; R-6 to P-160; A-7 to P-160; V-8 to P-160; L-9 to P-160; S-10 to P-160; A-11 to P-160; F-12 to P-160; H-13 to P-160; H-14 to P-160; T-15 to P-160; L-16 to P-160; Q-17 to P-160; L-18 to P-160; G-19 to P-160; P-20 to P-160; R-21 to P-160; E-22 to P-160; Q-23 to P-160; A-24 to P-160; R-25 to P-160; N-26 to P-160;

A-27 to P-160; S-28 to P-160; C-29 to P-160; P-30 to P-160; A-31 to P-160; G-32 to P-160; G-33 to P-160; R-34 to P-160; P-35 to P-160; A-36 to P-160; D-37 to P-160; R-38 to P-160; R-39 to P-160; F-40 to P-160; R-41 to P-160; P-42 to P-160; P-43 to P-160; T-44 to P-160; N-45 to P-160; L-46 to P-160; R-47 to P-160; S-48 to P-160; 5 V-49 to P-160; S-50 to P-160; P-51 to P-160; W-52 to P-160; A-53 to P-160; Y-54 to P-160; R-55 to P-160; I-56 to P-160; S-57 to P-160; Y-58 to P-160; D-59 to P-160; P-60 to P-160; A-61 to P-160; R-62 to P-160; Y-63 to P-160; P-64 to P-160; R-65 to P-160; Y-66 to P-160; L-67 to P-160; P-68 to P-160; E-69 to P-160; A-70 to P-160; Y-71 to P-160; C-72 to P-160; L-73 to P-160; C-74 to P-160; R-75 to P-160; G-76 to 10 P-160; C-77 to P-160; L-78 to P-160; T-79 to P-160; G-80 to P-160; L-81 to P-160; F-82 to P-160; G-83 to P-160; E-84 to P-160; E-85 to P-160; D-86 to P-160; V-87 to P-160; R-88 to P-160; F-89 to P-160; R-90 to P-160; S-91 to P-160; A-92 to P-160; P-93 to P-160; V-94 to P-160; Y-95 to P-160; M-96 to P-160; P-97 to P-160; T-98 to P-160; V-99 to P-160; V-100 to P-160; L-101 to P-160; R-102 to P-160; R-103 to 15 P-160; T-104 to P-160; P-105 to P-160; A-106 to P-160; C-107 to P-160; A-108 to P-160; G-109 to P-160; G-110 to P-160; R-111 to P-160; S-112 to P-160; V-113 to P-160; Y-114 to P-160; T-115 to P-160; E-116 to P-160; A-117 to P-160; Y-118 to P-160; V-119 to P-160; T-120 to P-160; I-121 to P-160; P-122 to P-160; V-123 to P-160; G-124 to P-160; C-125 to P-160; T-126 to P-160; C-127 to P-160; V-128 to 20 P-160; P-129 to P-160; E-130 to P-160; P-131 to P-160; E-132 to P-160; K-133 to P-160; D-134 to P-160; A-135 to P-160; D-136 to P-160; S-137 to P-160; I-138 to P-160; N-139 to P-160; S-140 to P-160; S-141 to P-160; I-142 to P-160; D-143 to P-160; K-144 to P-160; Q-145 to P-160; G-146 to P-160; A-147 to P-160; K-148 to P-160; L-149 to P-160; L-150 to P-160; L-151 to P-160; G-152 to P-160; P-153 to 25 P-160; N-154 to P-160; and D-155 to P-160 of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the 30 ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 and IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 and IL-22 proteins are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be 35 determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the

IL-21 polypeptide shown in SEQ ID NO:2, up to the aspartic acid residue at position 6 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the arginine residues at position 6 of SEQ ID NO:4. In particular, the present invention provides polypeptides having the amino acid sequence of residues 1-m<sup>3</sup> of the amino acid sequence in SEQ ID NO:2, where m<sup>3</sup> is any integer in the range of 6 to 87, and residue 5 is the position of the first residue from the C-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for immunogenic activity of the IL-21 protein. In addition, the present invention also provides polypeptides having the amino acid sequence of residues 1-m<sup>4</sup> of the amino acid sequence in SEQ ID NO:4, where m<sup>4</sup> is any integer in the range of 6 to 160, and residue 5 is the position of the first residue from the C-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for immunogenic activity of the IL-22 protein.

More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues A-1 to S-86; A-1 to R-85; A-1 to P-84; A-1 to L-83; A-1 to V-82; A-1 to C-81; A-1 to T-80; A-1 to C-79; A-1 to G-78; A-1 to V-77; A-1 to P-76; A-1 to V-75; A-1 to H-74; A-1 to I-73; A-1 to F-72; A-1 to E-71; A-1 to T-70; A-1 to H-69; A-1 to F-68; A-1 to A-67; A-1 to F-66; A-1 to A-65; A-1 to G-64; A-1 to P-63; A-1 to T-62; A-1 to P-61; A-1 to L-60; A-1 to G-59; A-1 to S-58; A-1 to G-57; A-1 to D-56; A-1 to R-55; A-1 to S-54; A-1 to C-53; A-1 to P-52; A-1 to R-51; A-1 to R-50; A-1 to R-49; A-1 to L-48; A-1 to V-47; A-1 to L-46; A-1 to L-45; A-1 to S-44; A-1 to Q-43; A-1 to L-42; A-1 to L-41; A-1 to R-40; A-1 to V-39; A-1 to S-38; A-1 to N-37; A-1 to L-36; A-1 to A-35; A-1 to A-34; A-1 to T-33; A-1 to E-32; A-1 to R-31; A-1 to G-30; A-1 to T-29; A-1 to R-28; A-1 to A-27; A-1 to D-26; A-1 to I-25; A-1 to C-24; A-1 to G-23; A-1 to R-22; A-1 to C-21; A-1 to L-20; A-1 to C-19; A-1 to E-18; A-1 to A-17; A-1 to F-16; A-1 to A-15; A-1 to L-14; A-1 to K-13; A-1 to Q-12; A-1 to P-11; A-1 to Y-10; A-1 to R-9; A-1 to D-8; A-1 to E-7; and A-1 to D-6 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

Moreover, the invention also provides polynucleotides encoding polypeptides having the amino acid sequence of residues N-1 to G-159; N-1 to A-158; N-1 to P-157; N-1 to A-156; N-1 to D-155; N-1 to N-154; N-1 to P-153; N-1 to G-152; N-1 to L-151; N-1 to L-150; N-1 to L-149; N-1 to K-148; N-1 to A-147; N-1 to G-146; N-1 to Q-145; N-1 to K-144; N-1 to D-143; N-1 to I-142; N-1 to S-141; N-1 to S-140; N-1 to N-139; N-1 to I-138; N-1 to S-137; N-1 to D-136; N-1 to A-135; N-1 to D-134; N-1 to K-133; N-1 to E-132; N-1 to P-131; N-1 to E-130; N-1 to P-129; N-1 to V-128; N-1

to C-127; N-1 to T-126; N-1 to C-125; N-1 to G-124; N-1 to V-123; N-1 to P-122;  
N-1 to I-121; N-1 to T-120; N-1 to V-119; N-1 to Y-118; N-1 to A-117; N-1 to E-116;  
N-1 to T-115; N-1 to Y-114; N-1 to V-113; N-1 to S-112; N-1 to R-111; N-1 to  
5 G-110; N-1 to G-109; N-1 to A-108; N-1 to C-107; N-1 to A-106; N-1 to P-105; N-1  
to T-104; N-1 to R-103; N-1 to R-102; N-1 to L-101; N-1 to V-100; N-1 to V-99; N-1  
to T-98; N-1 to P-97; N-1 to M-96; N-1 to Y-95; N-1 to V-94; N-1 to P-93; N-1 to  
A-92; N-1 to S-91; N-1 to R-90; N-1 to F-89; N-1 to R-88; N-1 to V-87; N-1 to D-86;  
N-1 to E-85; N-1 to E-84; N-1 to G-83; N-1 to F-82; N-1 to L-81; N-1 to G-80; N-1 to  
T-79; N-1 to L-78; N-1 to C-77; N-1 to G-76; N-1 to R-75; N-1 to C-74; N-1 to L-73;  
10 N-1 to C-72; N-1 to Y-71; N-1 to A-70; N-1 to E-69; N-1 to P-68; N-1 to L-67; N-1 to  
Y-66; N-1 to R-65; N-1 to P-64; N-1 to Y-63; N-1 to R-62; N-1 to A-61; N-1 to P-60;  
N-1 to D-59; N-1 to Y-58; N-1 to S-57; N-1 to I-56; N-1 to R-55; N-1 to Y-54; N-1 to  
A-53; N-1 to W-52; N-1 to P-51; N-1 to S-50; N-1 to V-49; N-1 to S-48; N-1 to R-47;  
N-1 to L-46; N-1 to N-45; N-1 to T-44; N-1 to P-43; N-1 to P-42; N-1 to R-41; N-1 to  
15 F-40; N-1 to R-39; N-1 to R-38; N-1 to D-37; N-1 to A-36; N-1 to P-35; N-1 to R-34;  
N-1 to G-33; N-1 to G-32; N-1 to A-31; N-1 to P-30; N-1 to C-29; N-1 to S-28; N-1 to  
A-27; N-1 to N-26; N-1 to R-25; N-1 to A-24; N-1 to Q-23; N-1 to E-22; N-1 to R-21;  
N-1 to P-20; N-1 to G-19; N-1 to L-18; N-1 to Q-17; N-1 to L-16; N-1 to T-15; N-1 to  
H-14; N-1 to H-13; N-1 to F-12; N-1 to A-11; N-1 to S-10; N-1 to L-9; N-1 to V-8;  
20 N-1 to A-7; and N-1 to R-6 of SEQ ID NO:4. Polynucleotides encoding these  
polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids  
deleted from both the amino and the carboxyl termini of IL-21, which may be described  
generally as having residues  $n^3$ - $m^3$  of SEQ ID NO:2, where  $n^3$  and  $m^3$  are integers as  
25 described above. Likewise, the invention also provides polypeptides having one or  
more amino acids deleted from both the amino and the carboxyl termini of IL-22, which  
may be described generally as having residues  $n^4$ - $m^4$  of SEQ ID NO:4, where  $n^4$  and  $m^4$   
are integers as described above.

Moreover, any polypeptide having one or more amino acids deleted from both  
30 the amino and the carboxyl termini of IL-22, described specifically as having residues  
 $n^4$ - $m^4$  of SEQ ID NO:4 (where  $n^4$  and  $m^4$  are integers as described above) may be  
excluded from the invention. In particular, any polypeptide having one or more amino  
acids deleted from both the amino and the carboxyl termini of IL-22 and which is  
defined by residues  $n^4$ - $m^4$  of SEQ ID NO:4, where  $n^4$  is equal to 21, 22, 23, 24 or 25  
35 and  $m^4$  is equal to 271, 272, 273, 274, 275 or 276 may be excluded from the invention.

Also as mentioned above, even if deletion of one or more amino acids from the  
N-terminus of a protein results in modification or loss of one or more biological

functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the full-length or mature IL-21 polypeptides generally will be retained when less than the majority of the residues of the full-length or mature IL-21 polypeptides are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete or full-length polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:29, up to the valine residue at position number 192, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n<sup>5</sup>-197 of SEQ ID NO:29, where n<sup>5</sup> is an integer in the range of 1 to 192, and 193 is the position of the first residue from the N-terminus of the full-length IL-21 polypeptide (shown in SEQ ID NO:29) believed to be required for immunogenic activity of the IL-21 protein.

More in particular, the invention provides polynucleotides encoding polypeptides comprising the amino acid sequence of residues T-2 to V-197; L-3 to V-197; L-4 to V-197; P-5 to V-197; G-6 to V-197; L-7 to V-197; L-8 to V-197; F-9 to V-197; L-10 to V-197; T-11 to V-197; W-12 to V-197; L-13 to V-197; H-14 to V-197; T-15 to V-197; C-16 to V-197; L-17 to V-197; A-18 to V-197; H-19 to V-197; H-20 to V-197; D-21 to V-197; P-22 to V-197; S-23 to V-197; L-24 to V-197; R-25 to V-197; G-26 to V-197; H-27 to V-197; P-28 to V-197; H-29 to V-197; S-30 to V-197; H-31 to V-197; G-32 to V-197; T-33 to V-197; P-34 to V-197; H-35 to V-197; C-36 to V-197; Y-37 to V-197; S-38 to V-197; A-39 to V-197; E-40 to V-197; E-41 to V-197; L-42 to V-197; P-43 to V-197; L-44 to V-197; G-45 to V-197; Q-46 to V-197; A-47 to V-197; P-48 to V-197; P-49 to V-197; H-50 to V-197; L-51 to V-197; L-52 to V-197; A-53 to V-197; R-54 to V-197; G-55 to V-197; A-56 to V-197; K-57 to V-197; W-58 to V-197; G-59 to V-197; Q-60 to V-197; A-61 to V-197; L-62 to V-197; P-63 to V-197; V-64 to V-197; A-65 to V-197; L-66 to V-197; V-67 to V-197; S-68 to V-197; S-69 to V-197; L-70 to V-197; E-71 to V-197; A-72 to V-197; A-73 to V-197; S-74 to V-197; H-75 to V-197; R-76 to V-197; G-77 to V-197; R-78 to V-197; H-79 to V-197; E-80 to V-197; R-81 to V-197; P-82 to V-197; S-83 to V-197; A-84 to V-197; T-85 to V-197; T-86 to V-197; Q-87 to V-197; C-88 to V-197; P-89 to V-197; V-90 to V-197; L-91 to V-197; R-92 to V-197; P-93 to V-197; E-94 to V-197; E-95 to V-197; V-96 to V-197; L-97 to V-197; E-98 to V-197; A-99 to V-197; D-100 to V-197; T-101 to V-197; H-102 to V-197; Q-103 to V-197; R-104 to V-197; S-105 to V-197; I-106 to V-197; S-107 to V-197; P-108 to V-197; W-109 to V-197; R-110 to V-197; Y-111 to V-197; R-112 to



More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues M-1 to S-196; M-1 to R-195; M-1 to P-194; M-1 to L-193; M-1 to V-192; M-1 to C-191; M-1 to T-190; M-1 to C-189; M-1 to G-188; M-1 to V-187; M-1 to P-186; M-1 to V-185; M-1 to H-184; M-1 to I-183; M-1 to F-182; M-1 to E-181; M-1 to T-180; M-1 to H-179; M-1 to F-178; M-1 to A-177; M-1 to F-176; M-1 to A-175; M-1 to G-174; M-1 to P-173; M-1 to T-172; M-1 to P-171; M-1 to L-170; M-1 to G-169; M-1 to S-168; M-1 to G-167; M-1 to D-166; M-1 to R-165; M-1 to S-164; M-1 to C-163; M-1 to P-162; M-1 to R-161; M-1 to R-160; M-1 to R-159; M-1 to L-158; M-1 to V-157; M-1 to L-156; M-1 to L-155; M-1 to S-154; M-1 to Q-153; M-1 to L-152; M-1 to L-151; M-1 to R-150; M-1 to V-149; M-1 to S-148; M-1 to N-147; M-1 to L-146; M-1 to A-145; M-1 to A-144; M-1 to T-143; M-1 to E-142; M-1 to R-141; M-1 to G-140; M-1 to T-139; M-1 to R-138; M-1 to A-137; M-1 to D-136; M-1 to I-135; M-1 to C-134; M-1 to G-133; M-1 to R-132; M-1 to C-131; M-1 to L-130; M-1 to C-129; M-1 to E-128; M-1 to A-127; M-1 to F-126; M-1 to A-125; M-1 to L-124; M-1 to K-123; M-1 to Q-122; M-1 to P-121; M-1 to Y-120; M-1 to R-119; M-1 to D-118; M-1 to E-117; M-1 to D-116; M-1 to T-115; M-1 to D-114; M-1 to V-113; M-1 to R-112; M-1 to Y-111; M-1 to R-110; M-1 to W-109; M-1 to P-108; M-1 to S-107; M-1 to I-106; M-1 to S-105; M-1 to R-104; M-1 to Q-103; M-1 to H-102; M-1 to T-101; M-1 to D-100; M-1 to A-99; M-1 to E-98; M-1 to L-97; M-1 to V-96; M-1 to E-95; M-1 to E-94; M-1 to P-93; M-1 to R-92; M-1 to L-91; M-1 to V-90; M-1 to P-89; M-1 to C-88; M-1 to Q-87; M-1 to T-86; M-1 to T-85; M-1 to A-84; M-1 to S-83; M-1 to P-82; M-1 to R-81; M-1 to E-80; M-1 to H-79; M-1 to R-78; M-1 to G-77; M-1 to R-76; M-1 to H-75; M-1 to S-74; M-1 to A-73; M-1 to A-72; M-1 to E-71; M-1 to L-70; M-1 to S-69; M-1 to S-68; M-1 to V-67; M-1 to L-66; M-1 to A-65; M-1 to V-64; M-1 to P-63; M-1 to L-62; M-1 to A-61; M-1 to Q-60; M-1 to G-59; M-1 to W-58; M-1 to K-57; M-1 to A-56; M-1 to G-55; M-1 to R-54; M-1 to A-53; M-1 to L-52; M-1 to L-51; M-1 to H-50; M-1 to P-49; M-1 to P-48; M-1 to A-47; M-1 to Q-46; M-1 to G-45; M-1 to L-44; M-1 to P-43; M-1 to L-42; M-1 to E-41; M-1 to E-40; M-1 to A-39; M-1 to S-38; M-1 to Y-37; M-1 to C-36; M-1 to H-35; M-1 to P-34; M-1 to T-33; M-1 to G-32; M-1 to H-31; M-1 to S-30; M-1 to H-29; M-1 to P-28; M-1 to H-27; M-1 to G-26; M-1 to R-25; M-1 to L-24; M-1 to S-23; M-1 to P-22; M-1 to D-21; M-1 to H-20; M-1 to H-19; M-1 to A-18; M-1 to L-17; M-1 to C-16; M-1 to T-15; M-1 to H-14; M-1 to L-13; M-1 to W-12; M-1 to T-11; M-1 to L-10; M-1 to F-9; M-1 to L-8; M-1 to L-7; and M-1 to G-6 of SEQ ID NO:29. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-21, which may be described



generally as having residues  $n^5$ - $m^5$  of SEQ ID NO:29, where  $n^5$  and  $m^5$  are integers as described above.

Moreover, any polypeptide having one or more amino acids deleted from both the amino and the carboxyl termini of IL-21, described specifically as having residues  $n^5$ - $m^5$  of SEQ ID NO:29 (where  $n^5$  and  $m^5$  are integers as described above) may be excluded from the invention.

The invention further includes IL-21 and IL-22 polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided by Bowie and colleagues (*Science* **247**:1306-1310 (1990)), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used (Cunningham and Wells, *Science* **244**:1081-1085 (1989)). The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of an aliphatic or hydrophobic amino acid with another aliphatic or hydrophobic amino acid such as Ala, Val, Leu or Ile; replacement of a hydroxyl residue with another hydroxyl residue such as Ser or Thr; replacement of an acidic residue with another acidic residue such as Asp or Glu; replacement of an amide residue with another amide residue such as Asn or Gln, replacement of a basic residue with another basic

residue such as Lys, Arg, or His; replacement of an aromatic residue with another aromatic residue such as Phe, Tyr, or Trp, and replacement of a small-sized amino acid with another small-sized residue such as Ala, Ser, Thr, Met, or Gly.

Besides conservative amino acid substitution, variants of IL-21 and IL-22 include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, IL-21 and IL-22 polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity (Pinckard, *et al.*, *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins, *et al.*, *Diabetes* 36:838-845 (1987); Cleland, *et al.*, *Crit. Rev. Ther. Drug Carrier Systems* 10:307-377 (1993)).

#### **Polynucleotide and Polypeptide Fragments**

The invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:3 which have been determined from the following related cDNA clones: HE2CD08R (SEQ ID NO:24); HAGBX04R (SEQ ID NO:25); HCEBA24FB (SEQ ID NO:26); and HCELE59R (SEQ ID NO:27). Furthermore, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:28 which has been determined from a related cDNA clone designated HTGED19RB (SEQ ID NO:30). Such polynucleotides (i.e., SEQ ID NOs:24, 25, 26, and 30) may preferably be excluded from the present invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence contained in the deposited clones or shown in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:28. The short nucleotide fragments are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20

or more contiguous bases from the cDNA sequence contained in the deposited clones or the nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:28. These nucleotide fragments are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of IL-21 polynucleotide fragments include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, or 701 to the end of SEQ ID NO:1 or the cDNA contained in the deposited clone. In addition, representative examples of IL-22 polynucleotide fragments include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1551-1600, or 1601 to the end of SEQ ID NO:3 or the cDNA contained in the deposited clone. Moreover, representative examples of the full-length IL-21 polynucleotide fragments include, for example, fragments having a sequence from about nucleotide number 1-1025, 50-1025, 100-1025, 150-1025, 200-1025, 250-1025, 300-1025, 350-1025, 400-1025, 450-1025, 500-1025, 550-1025, 600-1025, 650-1025, 700-1025, 750-1025, 800-1025, 850-1025, 900-1025, 950-1025, 1000-1025, 1-1000, 50-1000, 100-1000, 150-1000, 200-1000, 250-1000, 300-1000, 350-1000, 400-1000, 450-1000, 500-1000, 550-1000, 600-1000, 650-1000, 700-1000, 750-1000, 800-1000, 850-1000, 900-1000, 950-1000, 1-950, 50-950, 100-950, 150-950, 200-950, 250-950, 300-950, 350-950, 400-950, 450-950, 500-950, 550-950, 600-950, 650-950, 700-950, 750-950, 800-950, 850-950, 900-950, 1-900, 50-900, 100-900, 150-900, 200-900, 250-900, 300-900, 350-900, 400-900, 450-900, 500-900, 550-900, 600-900, 650-900, 700-900, 750-900, 800-900, 850-900, 1-850, 50-850, 100-850, 150-850, 200-850, 250-850, 300-850, 350-850, 400-850, 450-850, 500-850, 550-850, 600-850, 650-850, 700-850, 750-850, 800-850, 1-800, 50-800, 100-800, 150-800, 200-800, 250-800, 300-800, 350-800, 400-800, 450-800, 500-800, 550-800, 600-800, 650-800, 700-800, 750-800, 1-750, 50-750, 100-750, 150-750, 200-750, 250-750, 300-750, 350-750, 400-750, 450-750, 500-750, 550-750, 600-750, 650-750, 700-750, 1-700, 50-700, 100-700, 150-700, 200-700, 250-700, 300-700, 350-700, 400-700, 450-700, 500-700, 550-700, 600-700, 650-700, 1-650, 50-650, 100-650, 150-650, 200-650, 250-650, 300-650, 350-650, 400-650, 450-650, 500-650, 550-650, 600-650, 1-600, 50-600, 100-600, 150-600,

200-600, 250-600, 300-600, 350-600, 400-600, 450-600, 500-600, 550-600, 1-550, 50-550, 100-550, 150-550, 200-550, 250-550, 300-550, 350-550, 400-550, 450-550, 500-550, 1-500, 50-500, 100-500, 150-500, 200-500, 250-500, 300-500, 350-500, 400-500, 450-500, 1-450, 50-450, 100-450, 150-450, 200-450, 250-450, 300-450, 350-450, 400-450, 1-400, 50-400, 100-400, 150-400, 200-400, 250-400, 300-400, 350-400, 1-350, 50-350, 100-350, 150-350, 200-350, 250-350, 300-350, 1-300, 50-300, 100-300, 150-300, 200-300, 250-300, 1-250, 50-250, 100-250, 150-250, 200-250, 1-200, 50-200, 100-200, 150-200, 1-150, 50-150, 100-150, 1-100, 50-100, and 1-50 of SEQ ID NO:28. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity.

Further, the invention includes a polynucleotide comprising any portion of at least about 30 nucleotides, preferably at least about 50 nucleotides, of SEQ ID NO:1 from residue 1 to 650, 25 to 650, 50 to 650, 75 to 650, 100 to 650, 125 to 650, 150 to 650, 175 to 650, 200 to 650, 225 to 650, 250 to 650, 275 to 650, 300 to 650, 325 to 650, 350 to 650, 375 to 650, 400 to 650, 425 to 650, 500 to 650, 525 to 650, 550 to 650, 575 to 650, 600 to 650, 625 to 650, 1 to 600, 25 to 600, 50 to 600, 75 to 600, 100 to 600, 125 to 600, 150 to 600, 175 to 600, 200 to 600, 225 to 600, 250 to 600, 275 to 600, 300 to 600, 325 to 600, 350 to 600, 375 to 600, 400 to 600, 425 to 600, 500 to 600, 525 to 600, 550 to 600, 575 to 600, 1 to 550, 25 to 550, 50 to 550, 75 to 550, 100 to 550, 125 to 550, 150 to 550, 175 to 550, 200 to 550, 225 to 550, 250 to 550, 275 to 550, 300 to 550, 325 to 550, 350 to 550, 375 to 550, 400 to 550, 425 to 550, 500 to 550, 525 to 550, 1 to 500, 25 to 500, 50 to 500, 75 to 500, 100 to 500, 125 to 500, 150 to 500, 175 to 500, 200 to 500, 225 to 500, 250 to 500, 275 to 500, 300 to 500, 325 to 500, 350 to 500, 375 to 500, 400 to 500, 425 to 500, 450 to 500, 475 to 500, 1 to 450, 25 to 450, 50 to 450, 75 to 450, 100 to 450, 125 to 450, 150 to 450, 175 to 450, 200 to 450, 225 to 450, 250 to 450, 275 to 450, 300 to 450, 325 to 450, 350 to 450, 375 to 450, 400 to 450, 425 to 450, 1 to 400, 25 to 400, 50 to 400, 75 to 400, 100 to 400, 125 to 400, 150 to 400, 175 to 400, 200 to 400, 225 to 400, 250 to 400, 275 to 400, 300 to 400, 325 to 400, 350 to 400, 375 to 400, 1 to 350, 25 to 350, 50 to 350, 75 to 350, 100 to 350, 125 to 350, 150 to 350, 175 to 350, 200 to 350, 225 to 350, 250 to 350, 275 to 350, 300 to 350, 325 to 350, 1 to 300, 25 to 300, 50 to 300, 75 to 300, 100 to 300, 125 to 300, 150 to 300, 175 to 300, 200 to 300, 225 to 300, 250 to 300, 275 to 300, 1 to 250, 25 to 250, 50 to 250, 75 to 250, 100 to 250, 125 to 250, 150 to 250, 175 to 250, 200 to 250, 225 to 250, 1 to 200, 25 to 200, 50 to 200, 75 to 200, 100 to 200, 125 to 200, 150 to 200, 175 to 200, 1 to 150, 25 to 150,

50 to 150, 75 to 150, 100 to 150, 125 to 150, 1 to 100, 25 to 100, 50 to 100, 75 to 100, 1 to 50, and 25 to 50.

Moreover, the invention includes a polynucleotide comprising any portion of at least about 30 nucleotides, preferably at least about 50 nucleotides, of SEQ ID NO:3

5 from residue 300 to 850. More preferably, the invention includes a polynucleotide comprising nucleotide residues 50 to 850, 75 to 850, 100 to 850, 125 to 850, 150 to 850, 175 to 850, 200 to 850, 225 to 850, 250 to 850, 275 to 850, 300 to 850, 325 to 850, 350 to 850, 375 to 850, 400 to 850, 425 to 850, 450 to 850, 475 to 850, 500 to 850, 525 to 850, 550 to 850, 575 to 850, 600 to 850, 625 to 850, 650 to 850, 675 to 850, 700 to 850, 750 to 850, 775 to 850, 800 to 850, 50 to 800, 75 to 800, 100 to 800, 125 to 800, 150 to 800, 175 to 800, 200 to 800, 225 to 800, 250 to 800, 275 to 800, 300 to 800, 325 to 800, 350 to 800, 375 to 800, 400 to 800, 425 to 800, 450 to 800, 475 to 800, 500 to 800, 525 to 800, 550 to 800, 575 to 800, 600 to 800, 625 to 800, 650 to 800, 675 to 800, 700 to 800, 750 to 800, 50 to 750, 75 to 750, 100 to 750, 125 to 750, 150 to 750, 175 to 750, 200 to 750, 225 to 750, 250 to 750, 275 to 750, 300 to 750, 325 to 750, 350 to 750, 375 to 750, 400 to 750, 425 to 750, 450 to 750, 475 to 750, 500 to 750, 525 to 750, 550 to 750, 575 to 750, 600 to 750, 625 to 750, 650 to 750, 675 to 750, 700 to 750, 50 to 700, 75 to 700, 100 to 700, 125 to 700, 150 to 700, 175 to 700, 200 to 700, 225 to 700, 250 to 700, 275 to 700, 300 to 700, 325 to 700, 350 to 700, 375 to 700, 400 to 700, 425 to 700, 450 to 700, 475 to 700, 500 to 700, 525 to 700, 550 to 700, 575 to 700, 600 to 700, 625 to 700, 650 to 700, 50 to 650, 75 to 650, 100 to 650, 125 to 650, 150 to 650, 175 to 650, 200 to 650, 225 to 650, 250 to 650, 275 to 650, 300 to 650, 325 to 650, 350 to 650, 375 to 650, 400 to 650, 425 to 650, 450 to 650, 475 to 650, 500 to 650, 525 to 650, 550 to 650, 575 to 650, 600 to 650, 50 to 600, 75 to 600, 100 to 600, 125 to 600, 150 to 600, 175 to 600, 200 to 600, 225 to 600, 250 to 600, 275 to 600, 300 to 600, 325 to 600, 350 to 600, 375 to 600, 400 to 600, 425 to 600, 450 to 600, 475 to 600, 500 to 600, 525 to 600, 550 to 600, 50 to 550, 75 to 550, 100 to 550, 125 to 550, 150 to 550, 175 to 550, 200 to 550, 225 to 550, 250 to 550, 275 to 550, 300 to 550, 325 to 550, 350 to 550, 375 to 550, 400 to 550, 425 to 550, 450 to 550, 475 to 550, 500 to 550, 50 to 500, 75 to 500, 100 to 500, 125 to 500, 150 to 500, 175 to 500, 200 to 500, 225 to 500, 250 to 500, 275 to 500, 300 to 500, 325 to 500, 350 to 500, 375 to 500, 400 to 500, 425 to 500, 450 to 500, 50 to 450, 75 to 450, 100 to 450, 125 to 450, 150 to 450, 175 to 450, 200 to 450, 225 to 450, 250 to 450, 275 to 450, 300 to 450, 325 to 450, 350 to 450, 375 to 450, 400 to 450, 50 to 400, 75 to 400, 100 to 400, 125 to 400, 150 to 400, 175 to 400, 200 to 400, 225 to 400, 250 to 400, 275 to 400, 300 to 400, 325 to 400, 350 to 400, 50 to 350, 75 to 350, 100 to 350, 125 to 350,

350, 150 to 350, 175 to 350, 200 to 350, 225 to 350, 250 to 350, 275 to 350, 300 to 350, 50 to 300, 75 to 300, 100 to 300, 125 to 300, 150 to 300, 175 to 300, 200 to 300, 225 to 300, and 250 to 300.

In the present invention, a "polypeptide fragment" refers to a short amino acid sequence contained in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:29 or encoded by the cDNAs contained in the deposited clones. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the partial IL-21 invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-83 or to the end of the coding region. Moreover, polypeptide fragments of IL-21 can be about 10, 20, 30, 40, 50, 60, 70, or 80 amino acids in length. Representative examples of polypeptide fragments of the IL-22 invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 100-120, 120-140, 140-160, or to the end of the coding region. Moreover, polypeptide fragments of IL-22 can be about 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, or 150 amino acids in length. Representative examples of polypeptide fragments of the full-length IL-21 of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 100-120, 120-140, 140-160, 160-180, 180-200 or 180-to the end of the coding region. Moreover, polypeptide fragments of the full-length IL-21 can be about 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 150, 160, 170, 180 or 190 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted IL-21 and IL-22 proteins as well as the mature forms. Further preferred polypeptide fragments include the secreted IL-21 and IL-22 proteins or the mature forms having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted or the mature form of the IL-21 and IL-22 polypeptides. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted or the mature form of the IL-21 and IL-22 polypeptides. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these IL-21 and IL-22 polypeptide fragments are also preferred.

Also preferred are IL-21 and IL-22 polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions,

turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:29 falling within conserved domains are specifically contemplated by the present invention (Figures 4, 5, and 7). Moreover, polynucleotide fragments encoding these domains are also contemplated.

In additional embodiments, the polynucleotides of the invention encode functional attributes of IL-21 or IL-22. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of IL-21 or IL-22.

The data representing the structural or functional attributes of IL-21 set forth in Figure 7 and/or Table I, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. The data representing the structural or functional attributes of IL-22 set forth in Figure 5 and/or Table II, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. In a preferred embodiment, the data presented in columns VIII, IX, XIII, and XIV of Tables I and II can be used to determine regions of IL-21 and IL-22, respectively, which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or IV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Certain preferred regions in these regards are set out in Figures 5 and 7, but may, as shown in Tables II and I, respectively, be represented or identified by using tabular representations of the data presented in Figures 5 and 7, respectively. The DNA\*STAR computer algorithm used to generate Figures 5 and 7 (set on the original default parameters) was used to present the data in Figures 5 and 7 in a tabular format (See Tables II and I, respectively). The tabular format of the data in Figures 5 and 7 may be used to easily determine specific boundaries of a preferred region.

The above-mentioned preferred regions set out in Figures 5 and 7 and in Tables II and I, respectively, include, but are not limited to, regions of the aforementioned





Table I

	Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
5	Met	1	A	.	.	.	.	.	.	-0.80	0.76	.	.	.	-0.40	0.36
	Thr	2	.	.	B	.	.	.	.	-0.76	0.76	.	.	.	-0.40	0.44
	Leu	3	A	.	.	.	.	.	.	-1.18	0.76	.	.	.	-0.40	0.34
	Leu	4	A	.	.	.	.	T	.	-1.60	1.01	.	.	.	-0.20	0.28
	Pro	5	A	.	.	.	.	T	.	-1.91	1.09	.	.	F	-0.05	0.16
10	Gly	6	A	.	.	.	.	T	.	-2.12	1.39	.	.	.	-0.20	0.17
	Leu	7	A	.	.	.	.	T	.	-2.12	1.39	.	.	.	-0.20	0.17
	Leu	8	A	A	.	.	.	.	.	-1.60	1.19	.	.	.	-0.60	0.16
	Phe	9	A	A	.	.	.	.	.	-1.60	1.67	.	.	.	-0.60	0.17
15	Leu	10	A	A	.	.	.	.	.	-1.42	1.93	.	.	.	-0.60	0.17
	Thr	11	A	A	.	.	.	.	.	-1.39	1.74	.	.	.	-0.60	0.28
	Trp	12	A	A	.	.	.	.	.	-1.24	1.54	.	.	.	-0.60	0.46
	Leu	13	A	A	.	.	.	.	.	-1.24	1.33	.	.	.	-0.60	0.30
	His	14	A	A	.	.	.	.	.	-1.13	1.33	*	.	.	-0.60	0.17
20	Thr	15	A	A	.	.	.	.	.	-0.36	1.34	.	.	.	-0.60	0.17
	Cys	16	A	A	.	.	.	.	.	-0.08	0.93	.	.	.	-0.60	0.27
	Leu	17	A	A	.	.	.	.	.	0.21	0.74	.	.	.	-0.60	0.27
	Ala	18	.	A	.	.	T	.	.	0.81	0.24	.	.	.	0.10	0.32
	His	19	.	A	.	.	T	.	.	0.54	0.19	.	.	.	0.44	0.91
25	His	20	.	A	.	.	.	.	C	0.04	-0.00	.	*	.	1.33	1.48
	Asp	21	.	.	.	.	.	T	C	0.82	-0.00	.	*	F	2.22	1.21
	Pro	22	.	.	.	.	T	T	.	1.29	-0.50	.	*	F	2.76	1.74
	Ser	23	.	.	.	.	T	T	.	1.84	-0.57	.	*	F	3.40	1.27
	Leu	24	.	.	.	.	T	T	.	1.67	-0.57	.	*	F	3.06	1.03
30	Arg	25	.	.	.	.	T	.	.	1.67	-0.14	*	*	F	2.35	1.03
	Gly	26	.	.	.	.	T	.	.	1.37	-0.07	*	*	F	2.14	1.05
	His	27	.	.	.	.	.	T	C	1.54	-0.07	.	*	.	1.78	1.70
	Pro	28	.	.	.	.	.	T	C	1.50	-0.26	.	*	.	1.57	1.18
	His	29	.	.	.	.	T	T	.	2.00	0.17	.	*	.	1.30	1.18
35	Ser	30	.	.	.	.	T	T	.	1.68	0.23	.	*	.	1.17	1.26
	His	31	.	.	.	.	T	.	.	1.99	0.16	.	.	.	0.84	1.26
	Gly	32	.	.	.	.	T	.	.	1.36	0.23	.	.	F	0.86	1.26
	Thr	33	.	.	.	.	.	T	C	1.32	0.30	.	.	F	0.58	0.50
	Pro	34	.	.	.	.	.	T	C	1.06	0.67	.	.	F	0.15	0.58
40	His	35	.	.	.	.	T	T	.	0.77	0.56	.	.	.	0.20	0.78
	Cys	36	.	.	.	.	T	T	.	0.80	0.63	.	.	.	0.20	0.55
	Tyr	37	.	A	.	.	T	.	C	1.14	0.14	.	.	.	0.10	0.61
	Ser	38	.	A	.	.	.	.	C	0.64	-0.29	.	.	.	0.50	0.78
	Ala	39	A	A	.	.	.	.	.	0.64	-0.10	.	.	.	0.45	1.20
45	Glu	40	A	A	.	.	.	.	.	-0.13	-0.24	.	.	F	0.60	1.19
	Glu	41	A	A	.	.	.	.	.	0.19	-0.31	.	.	.	0.30	0.73
	Leu	42	A	.	.	.	.	T	.	0.43	-0.27	.	.	.	0.70	0.72
	Pro	43	A	.	.	.	.	T	.	0.14	-0.37	.	.	.	0.70	0.72
	Leu	44	.	.	.	.	T	T	.	0.52	0.13	.	.	.	0.50	0.42
50	Gly	45	.	.	.	.	T	T	.	0.31	0.56	.	.	F	0.35	0.78
	Gln	46	A	.	.	.	.	.	.	0.28	0.30	.	.	F	0.05	0.78
	Ala	47	.	.	.	.	.	.	C	0.28	0.37	*	.	F	0.40	1.29
	Pro	48	.	.	.	.	.	T	C	-0.32	0.37	*	.	F	0.60	1.08
	Pro	49	A	.	.	.	.	T	.	-0.10	0.63	*	*	F	-0.05	0.51
55	His	50	A	.	.	.	.	T	.	0.36	0.73	*	*	.	-0.20	0.51
	Leu	51	A	.	.	.	.	T	.	0.01	0.23	*	*	.	0.10	0.65
	Leu	52	A	A	.	.	.	.	.	0.01	0.23	*	*	.	-0.30	0.42
	Ala	53	A	A	.	.	.	.	.	0.27	0.30	*	.	.	-0.30	0.31
	Arg	54	A	A	.	.	.	.	.	0.19	-0.20	*	.	.	0.30	0.75
60	Gly	55	A	A	.	.	.	.	.	-0.12	0.03	*	.	F	-0.15	0.95
	Ala	56	A	A	.	.	.	.	.	0.69	-0.23	*	.	F	0.45	0.93

Table I (continued)

	Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
5	Lys	57	.	A	.	.	T	.	.	0.91	-0.33	*	.	F	0.85	0.83
	Trp	58	.	A	.	.	T	.	.	0.69	0.17	*	.	F	0.25	0.84
	Gly	59	.	A	.	.	.	.	C	0.37	0.43	*	.	F	-0.25	0.69
	Gln	60	A	A	.	.	.	.	.	-0.14	0.36	*	.	.	-0.30	0.53
	Ala	61	.	A	.	.	.	.	C	-0.14	1.00	*	.	.	-0.40	0.38
10	Leu	62	.	A	B	.	.	.	.	-1.00	0.59	*	.	.	-0.60	0.38
	Pro	63	.	A	B	.	.	.	.	-1.57	0.84	.	.	.	-0.60	0.18
	Val	64	A	A	.	.	.	.	.	-1.52	1.09	.	.	.	-0.60	0.13
	Ala	65	A	A	.	.	.	.	.	-1.82	0.97	.	.	.	-0.60	0.22
	Leu	66	A	A	.	.	.	.	.	-2.04	0.67	.	.	.	-0.60	0.19
15	Val	67	A	A	.	.	.	.	.	-1.23	0.93	.	.	.	-0.60	0.21
	Ser	68	A	A	.	.	.	.	.	-1.61	0.29	.	.	.	-0.30	0.36
	Ser	69	A	A	.	.	.	.	.	-1.34	0.29	.	.	.	-0.30	0.44
	Leu	70	A	A	.	.	.	.	.	-1.06	0.10	*	.	.	-0.30	0.60
	Glu	71	A	A	.	.	.	.	.	-0.28	-0.16	*	.	.	0.30	0.60
20	Ala	72	A	A	.	.	.	.	.	0.69	-0.04	*	.	.	0.30	0.61
	Ala	73	A	A	.	.	.	.	.	0.64	-0.43	*	*	.	0.79	1.45
	Ser	74	A	.	.	.	.	.	.	1.06	-0.69	*	*	.	1.48	0.83
	His	75	A	.	.	.	.	T	.	1.83	-0.69	*	*	.	2.17	1.60
	Arg	76	A	.	.	.	.	T	.	1.83	-0.69	.	*	F	2.66	2.16
25	Gly	77	.	.	.	.	T	T	.	2.53	-1.19	.	*	F	3.40	2.79
	Arg	78	.	.	.	.	T	T	.	2.91	-1.57	.	*	F	3.06	4.02
	His	79	.	.	.	.	.	.	C	2.91	-1.64	.	*	F	2.66	3.17
	Glu	80	.	.	.	.	.	.	C	2.36	-1.26	.	*	F	2.66	4.29
	Arg	81	.	.	.	.	.	T	C	1.93	-1.19	.	*	F	2.86	2.21
30	Pro	82	.	.	.	.	T	T	.	1.97	-0.70	.	.	F	3.06	2.35
	Ser	83	.	.	.	.	T	T	.	1.86	-0.71	.	*	F	3.40	1.96
	Ala	84	.	.	.	.	T	T	.	1.22	-0.31	.	*	F	2.76	1.73
	Thr	85	.	.	.	B	T	.	.	1.01	0.26	.	*	F	1.27	0.60
	Thr	86	.	.	.	B	T	.	.	0.04	0.26	.	*	F	0.93	0.69
35	Gln	87	.	.	.	B	T	.	.	-0.56	0.51	.	.	F	0.29	0.51
	Cys	88	.	.	B	B	.	.	.	-0.14	0.70	*	.	.	-0.60	0.29
	Pro	89	.	.	B	B	.	.	.	0.23	0.21	.	*	.	-0.30	0.39
	Val	90	.	.	.	B	.	.	C	0.54	0.16	.	.	.	-0.10	0.35
	Leu	91	.	A	.	.	.	.	C	0.86	-0.24	.	.	.	0.65	1.14
40	Arg	92	.	A	.	.	.	.	C	0.00	-0.81	*	.	F	1.10	1.27
	Pro	93	A	A	.	.	.	.	.	-0.14	-0.60	*	*	F	0.90	1.27
	Glu	94	A	A	.	.	.	.	.	0.07	-0.56	*	*	F	0.90	1.27
	Glu	95	A	A	.	.	.	.	.	0.33	-1.24	*	*	F	0.90	1.13
	Val	96	A	A	.	.	.	.	.	1.14	-0.74	.	*	.	0.60	0.74
45	Leu	97	A	A	.	.	.	.	.	0.72	-1.17	*	*	.	0.60	0.71
	Glu	98	A	A	.	.	.	.	.	0.90	-0.69	.	.	.	0.60	0.59
	Ala	99	A	A	.	.	.	.	.	0.90	-0.19	.	*	F	0.60	1.08
	Asp	100	A	.	.	.	.	T	.	1.01	-0.43	.	*	F	1.00	2.28
	Thr	101	A	.	.	.	.	T	.	1.57	-1.11	*	*	F	1.30	2.58
50	His	102	A	.	.	.	.	T	.	1.49	-0.73	*	*	F	1.30	3.42
	Gln	103	.	.	.	.	T	T	.	1.19	-0.54	*	.	F	1.91	1.43
	Arg	104	.	.	.	B	T	.	.	1.57	-0.16	*	.	F	1.42	1.33
	Ser	105	.	.	.	B	T	.	.	1.28	-0.21	*	*	F	1.63	1.51
	Ile	106	.	.	.	B	.	.	C	1.70	0.20	*	*	F	0.89	0.92
55	Ser	107	.	.	.	.	.	T	C	1.49	-0.20	*	*	F	2.10	0.92
	Pro	108	.	.	.	.	T	T	.	1.60	0.56	*	*	F	1.34	1.07
	Trp	109	.	.	.	.	T	T	.	0.63	0.17	*	*	.	1.28	3.00
	Arg	110	.	.	.	.	.	T	C	0.93	0.13	.	*	.	0.87	1.66
	Tyr	111	.	.	.	B	T	.	.	1.51	-0.26	.	*	.	1.40	1.80
60	Arg	112	.	.	.	B	T	.	.	1.81	-0.20	.	*	.	1.53	2.46

Table I (continued)

	Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
5	Val	113	.	.	.	B	.	.	C	2.02	-1.11	.	*	.	1.97	2.10
	Asp	114	.	.	.	.	T	T	.	2.31	-1.11	.	*	F	3.06	2.32
	Thr	115	.	.	.	.	T	T	.	2.31	-1.87	.	*	F	3.40	1.98
	Asp	116	.	.	.	.	T	T	.	2.31	-1.87	*	*	F	3.06	5.23
	Glu	117	.	.	.	.	T	T	.	1.99	-1.76	*	*	F	2.72	4.90
10	Asp	118	.	.	.	.	T	T	.	2.84	-1.33	*	.	F	2.38	5.25
	Arg	119	A	.	.	.	.	T	.	2.89	-1.41	*	*	F	1.64	5.45
	Tyr	120	A	.	.	.	.	T	.	2.39	-1.41	*	.	F	1.30	6.29
	Pro	121	A	.	.	.	.	T	.	1.80	-0.73	*	*	F	1.30	3.11
	Gln	122	A	A	.	.	.	.	.	1.10	-0.23	*	*	F	0.60	1.60
15	Lys	123	A	A	.	.	.	.	.	0.51	0.56	*	*	F	-0.45	0.89
	Leu	124	A	A	.	.	.	.	.	0.40	0.30	*	*	.	-0.30	0.58
	Ala	125	A	A	.	.	.	.	.	-0.02	-0.13	.	.	.	0.30	0.58
	Phe	126	A	A	.	.	.	.	.	-0.62	0.04	.	.	.	-0.30	0.16
	Ala	127	A	A	.	.	.	.	.	-1.29	0.73	*	.	.	-0.60	0.16
20	Glu	128	A	A	.	.	.	.	.	-1.22	0.61	*	*	.	-0.60	0.08
	Cys	129	A	A	.	.	.	.	.	-0.76	0.11	*	.	.	-0.30	0.19
	Leu	130	A	A	.	.	.	.	.	-0.83	-0.24	*	*	.	0.30	0.18
	Cys	131	.	.	.	.	T	T	.	-1.02	-0.17	*	*	.	1.10	0.06
	Arg	132	.	.	.	.	T	T	.	-0.43	0.51	*	*	.	0.20	0.07
25	Gly	133	.	.	.	.	T	T	.	-1.02	-0.06	*	*	.	1.10	0.15
	Cys	134	.	.	.	.	T	T	.	-0.24	-0.24	*	*	.	1.40	0.28
	Ile	135	A	.	.	.	.	.	.	0.26	-0.81	*	*	.	1.40	0.28
	Asp	136	.	.	.	.	T	.	.	0.58	-0.33	.	*	.	1.80	0.41
	Ala	137	.	.	.	.	T	.	.	0.58	-0.33	.	*	.	2.10	0.76
30	Arg	138	.	.	.	.	.	T	C	0.92	-0.90	*	*	F	3.00	2.12
	Thr	139	.	.	.	.	.	T	C	1.28	-1.59	*	*	F	2.70	2.20
	Gly	140	.	.	.	.	.	T	C	1.58	-1.10	*	*	F	2.40	3.14
	Arg	141	A	.	.	.	.	T	.	0.99	-1.10	*	.	F	1.90	1.62
	Glu	142	A	A	.	.	.	.	.	0.77	-0.60	*	*	F	1.20	1.13
35	Thr	143	A	A	.	.	.	.	.	0.66	-0.40	*	.	F	0.45	0.94
	Ala	144	A	A	.	.	.	.	.	0.67	-0.43	.	*	.	0.30	0.78
	Ala	145	A	A	.	.	.	.	.	0.16	-0.04	.	*	.	0.30	0.60
	Leu	146	A	.	.	B	.	.	.	0.16	0.60	.	*	.	-0.60	0.31
	Asn	147	A	.	.	B	.	.	.	-0.66	0.11	*	.	.	-0.30	0.60
40	Ser	148	A	.	.	B	.	.	.	-1.16	0.30	*	.	.	-0.30	0.49
	Val	149	A	.	.	B	.	.	.	-0.57	0.49	*	.	.	-0.60	0.49
	Arg	150	A	.	.	B	.	.	.	-0.28	0.20	*	.	.	-0.30	0.53
	Leu	151	A	.	.	B	.	.	.	-0.28	0.19	*	.	.	-0.30	0.53
	Leu	152	A	.	.	B	.	.	.	-1.09	0.49	*	*	.	-0.60	0.58
45	Gln	153	A	.	.	B	.	.	.	-1.64	0.53	*	*	.	-0.60	0.25
	Ser	154	A	.	.	B	.	.	.	-1.60	1.17	.	*	.	-0.60	0.22
	Leu	155	.	.	B	B	.	.	.	-1.60	1.17	*	.	.	-0.60	0.22
	Leu	156	.	.	B	B	.	.	.	-0.68	0.49	*	*	.	-0.60	0.25
	Val	157	.	.	B	B	.	.	.	0.24	0.09	*	*	.	-0.30	0.37
50	Leu	158	.	.	B	B	.	.	.	0.03	-0.30	*	.	.	0.30	0.87
	Arg	159	.	.	.	B	T	.	.	-0.33	-0.56	.	.	F	1.30	1.63
	Arg	160	.	.	.	B	T	.	.	0.18	-0.67	.	*	F	1.30	1.18
	Arg	161	.	.	.	B	.	.	C	1.10	-0.93	.	*	F	1.10	1.91
	Pro	162	.	.	.	.	T	.	.	1.96	-1.61	.	*	F	1.84	1.91
55	Cys	163	.	.	.	.	T	.	.	2.42	-1.61	.	*	F	2.18	1.63
	Ser	164	.	.	.	.	T	T	.	2.01	-1.19	.	*	F	2.57	0.82
	Arg	165	.	.	.	.	T	T	.	1.56	-0.80	*	.	F	2.91	0.71
	Asp	166	.	.	.	.	T	T	.	0.63	-0.80	*	*	F	3.40	1.32
	Gly	167	.	.	.	.	T	T	.	0.63	-0.69	*	.	F	2.91	0.81
60	Ser	168	.	.	.	.	T	.	.	0.99	-0.64	*	.	F	2.37	0.64

Table I (continued)

	Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
5	Gly	169	.	.	.	.	.	.	C	1.08	-0.16	*	.	F	1.53	0.55
	Leu	170	.	.	.	.	.	.	C	0.62	0.27	*	.	F	0.59	0.87
	Pro	171	.	.	.	.	.	.	C	0.03	0.27	.	.	F	0.25	0.64
	Thr	172	.	.	.	.	.	T	C	-0.32	0.39	.	.	F	0.45	0.65
10	Pro	173	.	.	.	.	.	T	C	-0.61	0.74	.	.	F	0.15	0.68
	Gly	174	.	.	.	.	.	T	C	-0.97	0.56	.	.	F	0.15	0.45
	Ala	175	A	.	.	.	.	T	.	-0.19	0.91	.	.	.	-0.20	0.27
	Phe	176	A	A	.	.	.	.	.	-0.29	0.93	.	.	.	-0.60	0.24
15	Ala	177	A	A	.	.	.	.	.	0.02	0.99	.	*	.	-0.60	0.34
	Phe	178	A	A	.	.	.	.	.	-0.47	0.56	.	.	.	-0.60	0.59
	His	179	A	A	.	.	.	.	.	-1.01	0.84	.	.	.	-0.60	0.59
	Thr	180	A	.	.	B	.	.	.	-0.46	0.74	.	.	.	-0.60	0.41
20	Glu	181	A	.	.	B	.	.	.	-0.61	0.74	.	.	.	-0.60	0.64
	Phe	182	A	.	.	B	.	.	.	-0.23	0.60	.	.	.	-0.60	0.35
	Ile	183	.	.	.	B	T	.	.	-0.39	0.53	.	.	.	-0.20	0.38
	His	184	.	.	.	B	T	.	.	-0.70	0.69	.	.	.	-0.20	0.16
25	Val	185	.	.	.	B	.	.	C	-1.06	1.11	.	.	.	-0.40	0.18
	Pro	186	.	.	.	.	T	T	.	-1.37	0.90	.	.	.	0.20	0.14
	Val	187	.	.	.	.	T	T	.	-1.33	0.70	.	.	.	0.20	0.15
	Gly	188	.	.	.	.	T	T	.	-1.30	0.77	.	*	.	0.20	0.11
30	Cys	189	.	.	.	.	T	T	.	-2.08	0.77	.	.	.	0.20	0.05
	Thr	190	.	.	B	B	.	.	.	-1.43	1.03	.	*	.	-0.60	0.06
	Cys	191	.	.	B	B	.	.	.	-1.11	0.81	.	.	.	-0.60	0.09
	Val	192	.	.	B	B	.	.	.	-0.56	0.39	*	.	.	-0.30	0.33
30	Leu	193	.	.	B	.	.	T	.	-1.07	0.20	*	.	.	0.28	0.31
	Pro	194	.	.	B	.	.	T	.	-0.79	0.36	*	.	F	0.61	0.42
	Arg	195	.	.	.	.	T	T	.	-0.87	0.21	*	.	.	1.04	0.73
	Ser	196	.	.	.	.	T	T	.	-0.59	-0.00	*	.	.	1.97	1.13
	Val	197	.	.	.	.	T	.	.	-0.12	-0.26	*	.	.	1.80	0.93

Table II

	Res	Position	I	II	III	IV	V	VI	VII	VIII		IX	X	XI	XII	XIII
5	Asn	1	.	.	.	.	.	.	C	0.58	.	*	.	0.85	1.60	
	Ser	2	.	A	.	.	.	.	C	1.08	.	*	.	0.65	1.26	
	Ala	3	.	A	B	.	.	.	.	0.88	.	*	.	0.75	1.93	
	Arg	4	.	A	B	.	.	.	.	0.41	.	*	.	0.75	1.22	
	Ala	5	.	A	B	.	.	.	.	-0.01	.	*	.	0.30	0.67	
10	Arg	6	.	A	B	.	.	.	.	-0.31	.	*	.	0.30	0.55	
	Ala	7	.	A	B	.	.	.	.	-0.60	.	*	.	0.30	0.38	
	Val	8	.	A	B	.	.	.	.	-0.71	.	*	.	-0.30	0.38	
	Leu	9	.	A	B	.	.	.	.	-0.86	*	*	.	-0.60	0.17	
	Ser	10	.	A	B	.	.	.	.	-0.30	*	*	.	-0.60	0.22	
15	Ala	11	.	A	B	.	.	.	.	-0.72	*	.	.	-0.60	0.41	
	Phe	12	.	A	B	.	.	.	.	-0.94	*	.	.	-0.60	0.72	
	His	13	.	A	B	.	.	.	.	-0.09	*	.	.	-0.60	0.44	
	His	14	.	A	B	.	.	.	.	-0.09	*	.	.	-0.60	0.76	
	Thr	15	.	A	B	.	.	.	.	-0.13	*	.	.	-0.60	0.72	
20	Leu	16	.	A	.	.	.	.	C	0.24	*	*	.	-0.10	0.52	
	Gln	17	.	A	.	.	T	.	.	1.06	*	*	.	0.40	0.60	
	Leu	18	.	A	.	.	.	.	C	1.09	.	*	.	0.80	0.81	
	Gly	19	.	.	.	.	.	T	C	1.12	.	*	F	2.40	1.70	
	Pro	20	.	.	.	.	.	T	C	0.84	*	*	F	3.00	1.70	
25	Arg	21	.	.	.	.	.	T	C	1.77	*	*	F	2.70	2.08	
	Glu	22	.	.	B	.	.	T	.	1.77	*	*	F	2.20	4.12	
	Gln	23	.	.	B	.	.	.	.	1.99	*	*	F	1.70	4.28	
	Ala	24	.	.	.	.	T	.	.	2.03	*	*	F	1.80	2.21	
	Arg	25	.	.	.	.	T	.	.	1.58	*	*	F	1.50	1.71	
30	Asn	26	.	.	.	.	T	.	.	1.26	*	*	F	1.05	0.53	
	Ala	27	.	.	.	.	T	.	.	0.67	*	.	.	0.90	0.81	
	Ser	28	.	.	B	.	.	.	.	0.32	.	.	.	0.78	0.42	
	Cys	29	.	.	B	.	.	T	.	0.57	.	*	.	0.66	0.26	
	Pro	30	.	.	.	.	T	T	.	0.57	.	*	.	1.34	0.25	
35	Ala	31	.	.	.	.	T	T	.	0.36	.	*	F	2.37	0.37	
	Gly	32	.	.	.	.	T	T	.	0.36	.	.	F	2.80	1.06	
	Gly	33	.	.	.	.	.	.	C	0.66	*	*	F	1.97	0.69	
	Arg	34	.	.	B	.	.	.	.	1.43	*	.	F	1.94	1.15	
	Pro	35	.	.	B	.	.	T	.	1.76	*	.	F	1.86	2.27	
40	Ala	36	.	.	B	.	.	T	.	1.64	*	*	F	1.58	4.49	
	Asp	37	.	.	B	.	.	T	.	2.10	*	*	F	1.30	1.99	
	Arg	38	.	.	B	.	.	T	.	2.23	*	*	F	1.30	2.52	
	Arg	39	.	.	B	.	.	.	.	1.91	*	*	F	1.10	3.85	
	Phe	40	.	.	B	.	.	.	.	1.81	*	*	F	1.44	3.57	
45	Arg	41	.	.	B	.	.	.	.	2.40	*	*	F	1.78	2.63	
	Pro	42	.	.	.	.	.	T	C	1.59	.	*	F	2.22	2.16	
	Pro	43	.	.	.	.	T	T	.	1.59	.	*	F	2.16	2.05	
	Thr	44	.	.	.	.	T	T	.	1.18	.	*	F	3.40	2.05	
	Asn	45	.	.	.	.	.	T	C	1.02	*	*	F	2.56	1.78	
50	Leu	46	.	.	B	B	.	.	.	0.61	*	*	F	0.87	0.85	
	Arg	47	.	.	B	B	.	.	.	0.61	*	.	F	1.13	0.79	
	Ser	48	.	.	B	B	.	.	.	0.53	*	.	F	0.79	0.76	
	Val	49	.	.	B	B	.	.	.	0.26	*	.	F	-0.45	0.97	
	Ser	50	.	.	B	.	.	T	.	0.01	*	*	F	0.25	0.50	
55	Pro	51	.	.	B	.	.	T	.	0.93	*	*	.	-0.20	0.59	
	Trp	52	.	.	B	.	.	T	.	-0.07	*	*	.	-0.05	1.55	
	Ala	53	.	.	B	.	.	T	.	-0.07	*	*	.	-0.20	0.81	
	Tyr	54	.	.	B	B	.	.	.	0.54	.	*	.	-0.60	0.70	
	Arg	55	.	.	B	B	.	.	.	0.84	.	*	.	-0.45	1.05	
60	Ile	56	.	.	B	B	.	.	.	0.84	*	*	.	0.13	1.73	

Table II (continued)

	Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
5	Ser	57	.	.	B	.	.	.	.	0.54	*	*	.	0.61	1.71
	Tyr	58	.	.	.	.	T	.	.	1.24	*	*	.	1.74	0.88
	Asp	59	.	.	.	.	.	T	C	1.24	*	*	F	2.32	2.46
	Pro	60	.	.	.	.	T	T	.	0.92	*	.	F	2.80	2.88
10	Ala	61	.	.	.	.	T	T	.	1.92	*	.	F	2.52	2.84
	Arg	62	.	.	B	.	.	T	.	1.98	*	.	F	2.14	3.33
	Tyr	63	.	.	B	.	.	T	.	1.41	*	.	.	1.41	3.37
	Pro	64	.	.	B	.	.	T	.	1.20	*	.	.	0.53	2.75
15	Arg	65	.	.	.	.	T	T	.	1.41	*	.	.	0.65	2.17
	Tyr	66	.	.	B	.	.	T	.	1.41	*	.	F	0.40	2.40
	Leu	67	.	.	B	.	.	.	.	1.06	*	.	F	0.80	1.57
	Pro	68	.	.	B	.	.	.	.	0.63	*	.	.	0.05	1.26
20	Glu	69	.	.	.	.	T	.	.	0.03	*	.	.	0.00	0.43
	Ala	70	.	.	B	B	.	.	.	-0.74	*	.	.	-0.60	0.43
	Tyr	71	.	.	B	B	.	.	.	-0.39	*	.	.	-0.60	0.15
	Cys	72	.	.	B	B	.	.	.	0.08	*	.	.	-0.30	0.17
25	Leu	73	.	.	B	B	.	.	.	-0.38	.	*	.	-0.60	0.16
	Cys	74	.	.	B	.	.	T	.	-1.19	.	*	.	-0.20	0.06
	Arg	75	.	.	B	.	.	T	.	-0.91	*	*	.	-0.20	0.09
	Gly	76	.	.	B	.	.	T	.	-1.01	*	.	.	-0.20	0.15
30	Cys	77	.	.	B	.	.	T	.	-1.16	.	*	.	0.10	0.28
	Leu	78	.	.	B	B	.	.	.	-1.04	.	.	.	-0.30	0.12
	Thr	79	.	.	B	B	.	.	.	-0.72	.	*	.	-0.60	0.10
	Gly	80	.	.	.	B	.	.	C	-0.83	.	*	.	-0.40	0.19
35	Leu	81	.	.	.	B	.	.	C	-0.49	.	.	.	-0.40	0.40
	Phe	82	.	.	B	B	.	.	.	0.18	.	.	F	0.45	0.48
	Gly	83	.	.	.	B	.	.	C	0.13	.	*	F	0.95	0.81
	Glu	84	.	A	B	.	.	.	.	0.56	.	*	F	0.45	0.73
40	Glu	85	.	A	B	.	.	.	.	0.20	.	*	F	0.90	1.65
	Asp	86	.	A	B	B	.	.	.	1.12	.	*	F	0.90	1.45
	Val	87	.	A	B	B	.	.	.	1.52	.	*	F	0.90	1.63
	Arg	88	.	A	.	B	T	.	.	1.28	.	*	.	1.15	1.26
45	Phe	89	.	A	.	B	T	.	.	1.07	.	*	.	1.00	0.77
	Arg	90	.	A	.	B	T	.	.	0.21	.	*	.	0.85	1.59
	Ser	91	.	A	.	B	.	.	C	-0.03	.	*	.	0.50	0.60
	Ala	92	.	.	.	B	.	.	C	0.22	.	*	.	-0.25	1.09
50	Pro	93	.	.	.	B	.	.	C	-0.10	.	*	.	-0.10	0.55
	Val	94	.	.	.	B	T	.	.	0.29	*	.	.	-0.20	0.64
	Tyr	95	.	.	B	B	.	.	.	-0.68	*	.	.	-0.60	0.91
	Met	96	.	.	B	B	.	.	.	-1.23	.	.	.	-0.60	0.44
55	Pro	97	.	.	B	B	.	.	.	-1.46	.	*	.	-0.60	0.44
	Thr	98	.	.	B	B	.	.	.	-1.13	*	.	.	-0.60	0.23
	Val	99	.	.	B	B	.	.	.	-0.17	*	.	.	-0.60	0.46
	Val	100	.	.	B	B	.	.	.	-0.23	.	.	.	0.30	0.58
60	Leu	101	.	.	B	B	.	.	.	0.16	.	.	.	0.30	0.58
	Arg	102	.	.	B	B	.	.	.	-0.22	.	.	F	0.60	1.20
	Arg	103	.	.	B	B	.	.	.	-0.58	.	.	F	0.60	1.63
	Thr	104	.	.	B	B	.	.	.	-0.31	.	.	F	0.60	1.06
55	Pro	105	.	.	B	B	.	.	.	0.20	*	.	F	1.00	0.55
	Ala	106	.	.	B	.	.	.	.	0.67	.	*	.	1.00	0.28
	Cys	107	.	.	B	.	.	T	.	0.67	.	.	.	0.85	0.19
	Ala	108	.	.	.	.	T	T	.	0.26	*	*	.	2.10	0.24
60	Gly	109	.	.	.	.	T	T	.	-0.29	*	.	F	2.50	0.32
	Gly	110	.	.	.	.	T	T	.	-0.32	*	.	F	2.25	0.44
	Arg	111	.	.	B	B	.	.	.	-0.04	*	.	F	0.60	0.69
	Ser	112	.	.	B	B	.	.	.	0.62	*	.	F	0.35	1.00

Table II (continued)

	Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
5	Val	113	.	.	B	B	.	.	.	0.62	*	.	.	0.70	1.75
	Tyr	114	.	.	B	.	.	.	.	0.72	.	.	.	0.50	0.90
	Thr	115	.	.	B	.	.	.	.	0.21	.	.	.	-0.25	1.05
	Glu	116	.	.	B	B	.	.	.	-0.21	.	*	.	-0.45	1.05
	Ala	117	.	.	B	B	.	.	.	-0.80	.	*	.	-0.60	0.97
10	Tyr	118	.	.	B	B	.	.	.	-0.16	.	*	.	-0.60	0.47
	Val	119	.	.	B	B	.	.	.	-0.77	.	*	.	-0.60	0.42
	Thr	120	.	.	B	B	.	.	.	-0.80	.	*	.	-0.60	0.31
	Ile	121	.	.	B	B	.	.	.	-1.47	.	*	.	-0.60	0.20
	Pro	122	.	.	B	.	.	T	.	-1.19	.	*	.	-0.20	0.14
15	Val	123	.	.	.	.	T	T	.	-1.61	.	.	.	0.20	0.14
	Gly	124	.	.	.	.	T	T	.	-1.61	.	.	.	0.20	0.11
	Cys	125	.	.	B	.	.	T	.	-1.51	.	.	.	-0.20	0.05
	Thr	126	.	.	B	.	.	.	.	-0.62	.	.	.	-0.40	0.11
	Cys	127	.	.	B	.	.	.	.	-0.62	.	.	.	-0.10	0.19
20	Val	128	.	.	B	.	.	T	.	0.23	.	.	.	0.40	0.55
	Pro	129	.	.	B	.	.	T	.	0.62	.	.	F	1.45	0.65
	Glu	130	.	.	B	.	.	T	.	1.29	*	.	F	2.20	2.44
	Pro	131	.	.	B	.	.	T	.	1.01	*	.	F	2.50	5.49
	Glu	132	.	.	.	.	T	.	.	1.68	*	.	F	3.00	3.59
25	Lys	133	A	.	.	.	.	.	.	2.23	*	.	F	2.30	3.46
	Asp	134	A	.	.	.	.	T	.	1.56	*	.	F	2.20	3.00
	Ala	135	A	.	.	.	.	T	.	1.56	*	.	F	1.90	1.21
	Asp	136	A	.	.	.	.	T	.	1.47	*	.	F	1.45	0.98
	Ser	137	.	.	B	.	.	T	.	1.17	*	.	F	1.15	0.78
30	Ile	138	.	.	B	.	.	.	.	0.23	*	.	F	0.80	1.04
	Asn	139	.	.	B	.	.	T	.	0.23	*	.	F	0.85	0.44
	Ser	140	.	.	B	.	.	T	.	0.87	*	.	F	1.16	0.54
	Ser	141	.	.	B	.	.	T	.	0.87	*	.	F	1.62	1.55
	Ile	142	.	.	B	.	.	T	.	0.82	.	*	F	2.23	1.67
35	Asp	143	.	.	B	.	.	T	.	1.12	*	*	F	2.54	1.23
	Lys	144	.	.	.	.	T	T	.	1.17	*	.	F	3.10	0.93
	Gln	145	.	.	B	.	.	T	.	0.66	*	.	F	2.54	2.65
	Gly	146	.	.	B	.	.	T	.	0.14	*	.	F	2.23	1.31
	Ala	147	.	A	B	.	.	.	.	0.22	*	.	F	1.07	0.54
40	Lys	148	.	A	B	.	.	.	.	-0.12	.	.	F	0.16	0.26
	Leu	149	.	A	B	.	.	.	.	-0.38	*	.	.	-0.60	0.26
	Leu	150	.	A	B	.	.	.	.	-0.38	.	.	.	-0.60	0.39
	Leu	151	.	A	B	.	.	.	.	-0.03	.	.	.	-0.06	0.32
	Gly	152	.	.	B	.	.	T	.	-0.03	.	.	F	0.73	0.64
45	Pro	153	.	.	.	.	.	T	C	-0.29	.	.	F	1.17	0.78
	Asn	154	.	.	.	.	T	T	.	-0.07	.	.	F	2.36	1.47
	Asp	155	.	.	.	.	.	T	C	0.40	.	.	F	2.40	1.50
	Ala	156	.	.	.	.	.	.	C	1.00	.	.	F	1.81	0.96
	Pro	157	.	.	.	.	.	T	C	0.96	.	.	F	1.77	0.92
50	Ala	158	.	.	.	.	.	T	C	0.78	.	.	.	1.38	0.71
	Gly	159	.	.	.	.	.	T	C	0.39	.	.	.	0.54	0.90
	Pro	160	.	.	B	.	.	T	.	0.00	.	.	.	0.10	0.74

Among highly preferred fragments in this regard are those that comprise regions of IL-21 or IL-22 that combine several structural features, such as several of the features set out above.

Other preferred fragments are biologically active IL-21 and IL-22 fragments.

- 5 Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the IL-21 and IL-22 polypeptides. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

## 10 Epitopes & Antibodies

- In the present invention, "epitopes" refer to IL-21 and IL-22 polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to an IL-21 or IL-22 polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this  
15 fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope". In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response (see, for instance, Geysen, *et al.*, *Proc. Natl. Acad. Sci. USA* **81**:3998- 4002 (1983)).

- Fragments which function as epitopes may be produced by any conventional  
20 means (see, e.g., Houghten, R. A., *Proc. Natl. Acad. Sci. USA* **82**:5131-5135 (1985); further described in U.S. Patent No. 4,631,211).

- In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including  
25 monoclonal antibodies, that specifically bind the epitope (see, for instance, Wilson, *et al.*, *Cell* **37**:767-778 (1984); Sutcliffe, J. G. *et al.*, *Science* **219**:660-666 (1983)).

- Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art (see, for instance, Sutcliffe, *et al.*, *supra*; Wilson, *et al.*, *supra*; Chow, M., *et al.*, *Proc. Natl. Acad. Sci. USA* **82**:910-914; and Bittle, F. J., *et al.*, *J. Gen. Virol.* **66**:2347-2354 (1985)). A preferred immunogenic epitope includes  
30 the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be  
35 sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).



Using DNASTar analysis, SEQ ID NO:2 was found antigenic at amino acids: from about Arg-2 to about Pro-11, from about Cys-24 to about Glu-32, and from about Arg-51 to about Gly-59. Thus, these regions can be used as epitopes to produce antibodies against the protein encoded by HTGED19. Again using DNASTar analysis, SEQ ID NO:4 was found antigenic at amino acids: from about Gly-19 to about Ala-27, from about Pro-30 to about Arg-38, from about Phe-40 to about Ser-48, from about Tyr-58 to about Leu-67, from about Pro-105 to about Val-113, from about Pro-129 to about Ser-137, from about Asn-139 to about Ala-147, and from about Leu-151 to about Gly-159. Thus, these regions can be used as epitopes to produce antibodies against the protein encoded by HFPBX96.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')<sub>2</sub> fragments) which are capable of specifically binding to protein. Fab and F(ab')<sub>2</sub> fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl, *et al.*, *J. Nucl. Med.* **24**:316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and humanized antibodies.

### **Fusion Proteins**

Any IL-21 or IL-22 polypeptide can be used to generate fusion proteins. For example, the IL-21 or IL-22 polypeptides, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the IL-21 or IL-22 polypeptides can be used to indirectly detect a second protein by binding to IL-21 or IL-22, respectively. Moreover, because secreted proteins target cellular locations based on trafficking signals, the IL-21 and IL-22 polypeptides can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to the IL-21 and IL-22 polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the IL-21 and IL-22 polypeptides. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the IL-21 and IL-22 polypeptides to improve stability and persistence during purification from the host cell or during subsequent handling and storage. Also, peptide moieties may be

added to the IL-21 and IL-22 polypeptides to facilitate purification. Such regions may be removed prior to final preparation of the IL-21 and IL-22 polypeptides. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

5           Moreover, IL-21 and IL-22 polypeptides, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various  
10 domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker, *et al.*, *Nature* **331**:84-86 (1988)). Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone (Fountoulakis, *et al.*, *J. Biochem.* **270**:3958-3964  
15 (1995)).

          Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a  
20 fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties (EP-A 0232 262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the  
25 purpose of high-throughput screening assays to identify antagonists of hIL-5 (see, Bennett, D., *et al.*, *J. Mol. Recog.* **8**:52-58 (1995); Johanson, K., *et al.*, *J. Biol. Chem.* **270**:9459-9471 (1995)).

          Moreover, the IL-21 and IL-22 polypeptides can be fused to marker sequences, such as a peptide which facilitates purification of IL-21 and IL-22, respectively. In  
30 preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described by Gentz and coworkers (*Proc. Natl. Acad. Sci. USA* **86**:821-824 (1989)), for instance, hexa-histidine provides for convenient purification of the fusion  
35 protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, *et al.*, *Cell* **37**:767 (1984)).

Thus, any of the above fusion proteins can be engineered using the IL-21 and IL-22 polynucleotides or the polypeptides.

### **Vectors, Host Cells, and Protein Production**

5       The present invention also relates to vectors containing the IL-21 and IL-22 polynucleotides, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

10       IL-21 and IL-22 polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

15       The IL-21 and IL-22 polynucleotide inserts should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

20       As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces*, and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

30       Among vectors preferred for use in bacteria include pHE4-5 and other pHE-like vectors; pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are

5 pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

10 Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals (for example, Davis, *et al.*, *Basic Methods In Molecular Biology* (1986)). It is specifically contemplated that IL-21 and IL-22 polypeptides may, in fact, be expressed by a host cell lacking a recombinant vector.

15 IL-21 and IL-22 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

20 IL-21 and IL-22 polypeptides, and preferably the secreted forms thereof, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the IL-21 and IL-22 polypeptides may be glycosylated or may be non-glycosylated. In addition, IL-21 and IL-22 polypeptides may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

#### Uses of the IL-21 and IL-22 Polynucleotides

35 The IL-21 and IL-22 polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Clone HTGED19 and clone HFPBX96 can each be mapped to a specific chromosome. Thus, IL-21 and IL-22 polynucleotides can then be used in linkage analysis as a marker for those specific chromosome.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:1 and SEQ ID NO:3. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human IL-21 or IL-22 genes corresponding to SEQ ID NO:1 or SEQ ID NO:3, respectively, will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the IL-21 and IL-22 polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the IL-21 and IL-22 polynucleotides can also be achieved using fluorescence *in situ* hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred (For review, see Verma, *et al.*, "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988)).

For chromosome mapping, the IL-21 and IL-22 polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

In a preferred embodiment, the gene encoding IL-22 of the present invention has been mapped using FISH technology to a location on human chromosome 13 at position 13q11.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a

particular disease (disease mapping data are found, for example, in McKusick, V., Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library)). Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the IL-21 and IL-22 polynucleotides and the corresponding genes between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the IL-21 and IL-22 polypeptides and the corresponding genes from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using IL-21 and IL-22 polynucleotides. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

In addition to the foregoing, an IL-21 or IL-22 polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Both methods rely on binding of the polynucleotide to DNA or RNA. For these techniques, preferred polynucleotides are usually 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee, *et al.*, *Nucl. Acids Res.* **6**:3073 (1979); Cooney, *et al.*, *Science* **241**:456 (1988); and Dervan, *et al.*, *Science* **251**:1360 (1991)) or to the mRNA itself (antisense - Okano, J. *Neurochem.* **56**:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease.

IL-21 and IL-22 polynucleotides are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. IL-21 and IL-22 offer means of targeting such

genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The IL-21 and IL-22 polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The IL-21 and IL-22 polynucleotides can be used as additional DNA markers for RFLP.

The IL-21 and IL-22 polynucleotides can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals (Erich, H., *PCR Technology*, Freeman and Co. (1992)). Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, IL-21 and IL-22 polynucleotides can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from IL-21 and IL-22 sequences. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

Because IL-21 is found expressed almost exclusively in apoptotic T-cells, IL-21 polynucleotides are useful as hybridization probes for differential identification of the

5 tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to IL-21 polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, for a number of disorders of the above tissues or cells, particularly of the Immune system, significantly higher or lower levels of IL-21 gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" IL-21 gene expression level, i.e., the IL-21 expression level in healthy tissue from an individual not having the Immune system disorder.

10 Likewise, since IL-22 is found expressed in bone marrow, skeletal muscle, and brain, IL-22 polynucleotides are useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to IL-22 polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, for a number of disorders of the above tissues or cells, particularly of the Immune system, significantly higher or lower levels of IL-22 gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" IL-22 gene expression level, i.e., the IL-22 expression level in healthy tissue from an individual not having the Immune system disorder.

20 Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying IL-21 or IL-22 gene expression level in cells or body fluid of an individual; (b) comparing the IL-21 or IL-22 gene expression level with a standard IL-21 or IL-22 gene expression level, respectively, whereby an increase or decrease in the assayed IL-21 or IL-22 gene expression level compared to the standard expression level is indicative of disorder in the Immune system.

25 In the very least, the IL-21 and IL-22 polynucleotides can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

### 35 Uses of IL-21 and IL-22 Polypeptides

IL-21 and IL-22 polypeptides can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.



IL-21 and IL-22 polypeptides can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al.*, *J. Cell. Biol.* **101**:976-985 (1985); Jalkanen, M., *et al.*, *J. Cell. Biol.* **105**:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected *in vivo* by imaging. Antibody labels or markers for *in vivo* imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described by Burchiel and colleagues ("Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of IL-21 or IL-22 polypeptides in cells or body fluid of an individual; (b) comparing the level of IL-21 or IL-22 gene expression with a standard gene expression level, whereby an increase or decrease in the assayed IL-21 or IL-22

polypeptide gene expression level compared to the standard expression level is indicative of a disorder.

Moreover, IL-21 and IL-22 polypeptides can be used to treat disease. For example, patients can be administered IL-21 and IL-22 polypeptides in an effort to replace absent or decreased levels of the IL-21 and IL-22 polypeptides, respectively, (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B), to inhibit the activity of a polypeptide (e.g., an oncogene), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth).

Similarly, antibodies directed to IL-21 and IL-22 polypeptides can also be used to treat disease. For example, administration of an antibody directed to an IL-21 or IL-22 polypeptide can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the IL-21 and IL-22 polypeptides can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. IL-21 and IL-22 polypeptides can also be used to raise antibodies, which, in turn, are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, IL-21 and IL-22 polypeptides can be used to test the following biological activities.

#### **Biological Activities of IL-21 and IL-22**

IL-21 and IL-22 polynucleotides and polypeptides can be used in assays to test for one or more biological activities. If IL-21 and IL-22 polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that IL-21 and IL-22 may be involved in the diseases associated with the biological activity. Therefore, IL-21 and IL-22 could be used to treat the associated disease.

The IL-21 and IL-22 proteins of the present invention modulate IL-6 secretion from NIH-3T3 cells. An *in vitro* ELISA assay which quantitates the amount of IL-6 secreted from cells in response to treatment with cytokines or the soluble extracellular domains of cytokine receptors has been described (Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). Briefly, the assay involves plating the target cells at a density of approximately  $5 \times 10^6$  cells/mL in a volume of 500  $\mu$ L in the wells of a 24 well flat-bottomed culture plate (Costar). The cultures are then treated with various concentrations of the cytokine or the soluble extracellular domain of cytokine receptor in

question. The cells are then cultured for 24 hours at 37°C. At this time, 50 µL of supernatant is removed and assayed for the quantity of IL-6 essentially as described by the manufacturer (Genzyme, Boston, MA). IL-6 levels are then calculated by reference to a standard curve constructed with recombinant IL-17 cytokine. Such activity is useful for determining the level of IL-21- or IL-22-mediated IL-6 secretion.

IL-21 and IL-22 protein modulates immune system cell proliferation and differentiation in a dose-dependent manner in the above-described assay. Thus, "a polypeptide having IL-21 or IL-22 protein activity" includes polypeptides that also exhibit any of the same stimulatory activities in the above-described assays in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the IL-21 or IL-22 proteins, preferably, "a polypeptide having IL-21 or IL-22 protein activity" will exhibit substantially similar dose-dependence in a given activity as compared to the IL-21 or IL-22 protein (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity relative to the reference IL-21 or IL-22 protein).

Lymphocyte proliferation is another *in vitro* assay which may be performed to determine the activity of IL-21 and IL-22. For example, Yao and colleagues (*Immunity* 3:811-821 (1995)) have recently described an *in vitro* assay for determining the effects of various cytokines and soluble cytokine receptors on the proliferation of murine leukocytes. Briefly, lymphoid organs are harvested aseptically, lymphocytes are isolated from the harvested organs, and the resulting collection of lymphoid cells are suspended in standard culture medium as described by Fanslow and coworkers (*J. Immunol.* 147:535-5540 (1991)). The lymphoid cell suspensions may then be divided into several different subclasses of lymphoid cells including splenic T-cells, lymph node B-cells, CD4<sup>+</sup> and CD8<sup>+</sup> T-cells, and mature adult thymocytes. For splenic T-cells, spleen cell suspensions (200 x 10<sup>6</sup> cells) are incubated with CD11b mAb and class II MHC mAb for 30 min at 4°C, loaded on a T-cell purification column (Pierce, Rockford, IL), and the T-cells eluted according to the manufacturer's instructions. Using this method, purity of the resulting T-cell populations should be >95% CD3<sup>+</sup> and <1% sIgM<sup>+</sup>. For purification of lymph node subsets, B-cells are removed from by adherence to tissue culture dishes previously coated with goat anti-mouse IgG (10µg/mL). Remaining cells were then incubated with anti-CD4 or anti-CD8 for 30 min at 4°C then washed and placed on tissue culture dishes previously coated with goat anti-rat IgG (20 µg/mL). After 45 min, nonadherent cells are removed and tested for purity by flow cytometry. CD4 and surface Ig-depleted cells should be >90% TCR-ab, CD8<sup>+</sup>, whereas CD8 and surface Ig-depleted cells should be >95% TCR-ab, CD4<sup>+</sup>. Finally, to enrich for mature adult thymocytes, cells are suspended at 10<sup>8</sup>/mL in 10%

anti-HSA and 10% low tox rabbit complement (Cedarlane, Ontario, Canada), incubated for 45 min at 37°C, and remaining viable cells isolated over Ficoll-Hypaque (Pharmacia, Piscataway, NJ). This procedure should yield between 90 and 95% CD3<sup>+</sup> cells that are either CD4<sup>+</sup> or CD4<sup>+</sup>8<sup>+</sup>.

5

### **Immune Activity**

IL-21 and IL-22 polypeptides or polynucleotides may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, IL-21 and IL-22 polynucleotides or polypeptides can be used as a marker or detector of a particular immune system disease or disorder.

IL-21 and IL-22 polynucleotides or polypeptides may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. IL-21 and IL-22 polypeptides or polynucleotides could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, IL-21 and IL-22 polypeptides or polynucleotides can also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, IL-21 and IL-22 polynucleotides or polypeptides could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, IL-21 and IL-22 polynucleotides or polypeptides that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting, important in the treatment of heart attacks (infarction), strokes, or scarring.

IL-21 and IL-22 polynucleotides or polypeptides may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of IL-21 and IL-22 polypeptides or polynucleotides that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected by IL-21 and IL-22 include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by IL-21 and IL-22 polypeptides or polynucleotides. Moreover, IL-21 and IL-22 can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

IL-21 and IL-22 polynucleotides or polypeptides may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of IL-21 and IL-22 polypeptides or polynucleotides that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, IL-21 and IL-22 polypeptides or polynucleotides may also be used to modulate inflammation. For example, IL-21 and IL-22 polypeptides or polynucleotides may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury,

inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

### **Hyperproliferative Disorders**

IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect hyperproliferative disorders, including neoplasms. IL-21 and IL-22 polypeptides or polynucleotides may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, IL-21 and IL-22 polypeptides or polynucleotides may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

### **Infectious Disease**

IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, IL-21 and

IL-22 polypeptides or polynucleotides may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides. Examples of viruses, include, but are not limited to the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. IL-21, and IL-22 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides include, but not limited to, the following Gram-Negative and Gram-positive bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, Enterobacteriaceae (Klebsiella, Salmonella, Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Meningococcal), Pasteurellacea Infections (e.g., Actinobacillus, Haemophilus, Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, and Staphylococcal. These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme

Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis, Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

Moreover, parasitic agents causing disease or symptoms that can be treated or detected by IL-21 polynucleotides or polypeptides include, but not limited to, the following families: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas. These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), Malaria, pregnancy complications, and toxoplasmosis. IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

Preferably, treatment using IL-21 and IL-22 polypeptides or polynucleotides could either be by administering an effective amount of IL-21 or IL-22 polypeptide to the patient, or by removing cells from the patient, supplying the cells with IL-21 and IL-22 polynucleotide, and returning the engineered cells to the patient (*ex vivo* therapy). Moreover, the IL-21 and IL-22 polypeptide or polynucleotide can be used as an antigen in a vaccine to raise an immune response against infectious disease.

### **Regeneration**

IL-21 and IL-22 polynucleotides or polypeptides can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues (see, *Science* 276:59-87 (1997)). The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vascular (including vascular endothelium), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.



Moreover, IL-21 and IL-22 polynucleotides or polypeptides may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. IL-21 and IL-22 polynucleotides or polypeptides of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using IL-21 and IL-22 polynucleotides or polypeptides to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the IL-21 and IL-22 polynucleotides or polypeptides.

### **Chemotaxis**

IL-21 and IL-22 polynucleotides or polypeptides may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

IL-21 and IL-22 polynucleotides or polypeptides may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. As a chemotactic molecule, IL-21 and IL-22 could also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that IL-21 and IL-22 polynucleotides or polypeptides may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, IL-21 and IL-22 polynucleotides or polypeptides could be used as an inhibitor of chemotaxis.

### **Binding Activity**

IL-21 and IL-22 polypeptides may be used to screen for molecules that bind to IL-21 or IL-22 or for molecules to which IL-21 or IL-22 bind. The binding of IL-21 and IL-22 and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the IL-21 and IL-22 or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of IL-21 or IL-22, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic (see, Coligan, *et al.*, Current Protocols in Immunology 1(2):Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural receptor to which IL-21 and IL-22 bind, or at least, a fragment of the receptor capable of being bound by IL-21 or IL-22 (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express IL-21 and IL-22, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing IL-21 and IL-22 (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either IL-21 and IL-22 or the molecule.

The assay may simply test binding of a candidate compound to IL-21 or IL-22, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to IL-21 or IL-22.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing IL-21 or IL-22, measuring IL-21/molecule or IL-22/molecule activity or binding, respectively, and comparing the IL-21/molecule or IL-22/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure IL-21 and IL-22 levels or activities in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure IL-21 and IL-22 levels or activities by either binding, directly or indirectly, to IL-21 or IL-22 or by competing with IL-21 or IL-22 for a substrate.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a

particular result in a patient (e.g., blood vessel growth) by activating or inhibiting IL-21 or IL-22. Moreover, the assays can discover agents which may inhibit or enhance the production of IL-21 and IL-22 from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which  
5 bind to IL-21 and IL-22 comprising the steps of: (a) incubating a candidate binding compound with IL-21 or IL-22; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with IL-21 or IL-22, (b) assaying a biological activity, and (b) determining if a biological activity of IL-21 or  
10 IL-22, respectively, has been altered.

### **Other Activities**

IL-21 and IL-22 polypeptides or polynucleotides may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above,  
15 hematopoietic lineage.

IL-21 and IL-22 polypeptides or polynucleotides may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, IL-21 and IL-22 polypeptides or polynucleotides may be used to modulate  
20 mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

IL-21 and IL-22 polypeptides or polynucleotides may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain,  
25 reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

IL-21 and IL-22 polypeptides or polynucleotides may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional  
30 components.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

### **Examples**

5 In the case where the full-length IL-21 and the partial IL-22 are not specifically mentioned, specific details are provided in the following examples only for the partial-length IL-21 molecules of the present invention. However, the examples can also be easily performed for the full-length IL-21 and the full-length or partial-length IL-22 molecules of the present invention by using the details provided for the partial IL-21 and substituting appropriate nucleotides or amino acid residues of the full-length IL-21, the full-length or partial-length IL-22, and/or any deletion mutations or other variants of either IL-21 or IL-22, for example, in the design of suitable PCR primers, and the like. The use or applicability of another IL-21 or IL-22 in place of the IL-21 exemplified below is thus contemplated in each of the following examples. When provided with the nucleotide and amino acid sequences of IL-21 (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:28, and SEQ ID NO:29) and IL-22 (SEQ ID NO:3 and SEQ ID NO:4) of the present invention, one of ordinary skill in the art could easily perform the following examples with the intent of isolating or further characterizing or manipulating another IL-21 or IL-22 in place of the IL-21 shown in the Examples below.

**Example 1: Isolation of the IL-21 and IL-22 cDNA Clones From the Deposited Samples**

20 The cDNAs encoding the partial IL-21 and IL-22 molecules are each inserted into the *Eco* RI and *Xho* I restriction sites of the multiple cloning site of pBluescript. pBluescript contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies (see, for instance, Gruber, C. E., et al., *Focus* 15:59 (1993)).

25 Two approaches can be used to isolate IL-21 from the deposited sample. First, a specific polynucleotide of SEQ ID NO:1 with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with <sup>32</sup>P-γ-ATP using T4 polynucleotide kinase and purified according to routine methods (e.g., Maniatis, *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982)). The plasmid mixture is transformed into a suitable host (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g.,

Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the  
5 SEQ ID NO:1 (i.e., within the region of SEQ ID NO:1 bounded by the 5' and 3'  
nucleotides of the clone) are synthesized and used to amplify the IL-21 cDNA using the  
deposited cDNA plasmid as a template. The polymerase chain reaction is carried out  
under routine conditions, for instance, in 25  $\mu$ l of reaction mixture with 0.5  $\mu$ g of the  
above cDNA template. A convenient reaction mixture is 1.5-5 mM  $MgCl_2$ , 0.01%  
10 (w/v) gelatin, 20  $\mu$ M each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and  
0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1  
min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a  
Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by  
agarose gel electrophoresis and the DNA band with expected molecular weight is  
15 excised and purified. The PCR product is verified to be the selected sequence by  
subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding  
portions of the IL-21 gene which may not be present in the deposited clone. These  
methods include, but are not limited to, filter probing, clone enrichment using specific  
20 probes, and protocols similar or identical to 5' and 3' RACE protocols which are well  
known in the art. For instance, a method similar to 5' RACE is available for generating  
the missing 5' end of a desired full-length transcript (Fromont-Racine, *et al.*, *Nucl.*  
*Acids Res.* **21**(7):1683-1684 (1993)).

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population  
25 of RNA presumably containing full-length gene RNA transcripts. A primer set  
containing a primer specific to the ligated RNA oligonucleotide and a primer specific to  
a known sequence of the IL-21 gene of interest is used to PCR amplify the 5' portion of  
the IL-21 full-length gene. This amplified product may then be sequenced and used to  
generate the full length gene.

30 This above method starts with total RNA isolated from the desired source,  
although poly-A+ RNA can be used. The RNA preparation can then be treated with a  
phosphatase, if necessary, to eliminate 5' phosphate groups on degraded or damaged  
RNA which may interfere with the later RNA ligase step. The phosphatase should then  
be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to  
35 remove the cap structure present at the 5' ends of messenger RNA. This reaction leaves  
a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to  
an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the IL-21 gene.

### **Example 2: Isolation of IL-21 Genomic Clones**

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:1., according to the method described in Example 1 (see also, Sambrook, *et al.*, *supra*).

### **Example 3: Tissue Distribution of IL-21**

Tissue distribution of mRNA expression of IL-21 is determined using protocols for Northern blot analysis, described by, among others, Sambrook and colleagues (*supra*). For example, an IL-21 probe produced by the method described in Example 1 is labeled with <sup>32</sup>P using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system (IM) tissues (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and the films developed according to standard procedures.

Using essentially the above-prescribed protocol, Northern blot analyses were performed to determine the expression pattern of IL-21 and IL-22. In the case of IL-21, a major message of approximately 5 kb was detected predominantly in thymus, but was also detectable in adrenal cortex, spleen, pancreas, and very weakly in lymph node, PBL, fetal liver, adrenal medulla, thyroid, small intestine, stomach, and heart. In the case of IL-22, a major message of slightly less than 1 kb was detected in

conjunction with a minor band of approximately 5 kb predominantly in testis and spinal cord, but was also detected in bone marrow and small intestine.

#### 5 **Example 4: Chromosomal Mapping of IL-21**

10 An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:1. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions are analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is  
15 determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

#### 20 **Example 5: Bacterial Expression of IL-21**

An IL-21 polynucleotide encoding an IL-21 polypeptide of the invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites,  
25 such as *Bam* HI and *Hin* dIII, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, *Bam* HI and *Hin* dIII correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (*Amp*<sup>R</sup>), a bacterial origin of replication (*ori*), an IPTG-regulatable promoter/operator  
30 (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

Specifically, to clone the mature domain of the IL-21 protein in a bacterial vector, the 5' primer has the sequence 5'-GAT CGC GGA TCC GAC ACG GAT GAG GAC CGC TAT CCA CAG AAG CTG-3' (SEQ ID NO:9) containing the underlined  
35 *Bam* HI restriction site followed several nucleotides of the amino terminal coding sequence of the mature IL-21 sequence in SEQ ID NO:1. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the

5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete IL-21 protein shorter or longer than the mature form of the protein. The 3' primer has the sequence 5'-CCC AAG CTT TCA CAC TGA ACG GGG CAG CAC GCA GGT GCA GC-3' (SEQ ID NO:10) containing the underlined *Hin* dIII restriction site followed by a number nucleotides complementary to the 3' end of the coding sequence of the IL-21 DNA sequence of SEQ ID NO:1.

The pQE-9 vector is digested with *Bam* HI and *Hin* dIII and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the *E. coli* strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the *lacI* repressor and also confers kanamycin resistance (Kan<sup>R</sup>). Transformants are identified by their ability to grow on LB plates and colonies are selected which are resistant to both ampicillin and kanamycin. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 µg/ml) and Kan (25 µg/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sub>600</sub>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the *lacI* repressor, clearing the promoter/operator leading to increased gene expression.

Cells are grown for an additional 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000 X g). The cell pellet is solubilized in the chaotropic agent 6 M Guanidine-HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified IL-21 protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the IL-21 protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a



linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified IL-21 protein is stored at 4° C or frozen at -80° C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to an IL-21 polynucleotide, called pHE4a (ATCC Accession Number 209645, deposited February 25, 1998). This vector contains: (1) a neomycin phosphotransferase gene as a selection marker, (2) an *E. coli* origin of replication, (3) a T5 phage promoter sequence, (4) two lac operator sequences, (5) a Shine-Delgarno sequence, and (6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with *Nde* I and *Xba* I, *Bam* HI, *Xho* I, or *Asp* 718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers which encode restriction sites for *Nde* I (5' primer) and *Nde* I and *Xba* I, *Bam* HI, *Xho* I, or *Asp* 718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

#### **Example 6: Purification of IL-21 Polypeptide from an Inclusion Body**

The following alternative method can be used to purify IL-21 polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10° C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10° C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50

mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 x g centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 x g) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 µm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the IL-21 polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant IL-21 polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 µg of purified

protein is loaded. The purified IL-21 protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

5

#### **Example 7: Cloning and Expression of IL-21 in a Baculovirus Expression System**

10 In this example, the plasmid shuttle vector pA2 is used to insert IL-21 polynucleotide into a baculovirus to express IL-21. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as *Bam* HI, *Xba* I and *Asp* 718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the  
15 beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned IL-21 polynucleotide.

20 Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, by Luckow and colleagues  
25 (*Virology* 170:31-39 (1989)).

Specifically, the IL-21 cDNA sequence contained in the deposited clone, including the AUG initiation codon and any naturally associated leader sequence, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a  
30 second signal peptide. However, since the predicted naturally occurring signal peptides of IL-21 and IL-22 are not known, the vector can be modified (now designated pA2GP) to include a baculovirus leader sequence, using the standard methods described by Summers and coworkers ("A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin  
35 No. 1555 (1987)).

More specifically, the cDNA sequence encoding the full-length IL-21 protein in the deposited clone is amplified using PCR oligonucleotide primers corresponding to

the 5' and 3' sequences of the gene. The 5' primer has the sequence 5'-CGC CGC GGA TCC *GCC ATC* CGC ACG AGT GGA CAC GG-3' (SEQ ID NO:11) containing the *Bam* HI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells (shown in the primer sequence in italics; Kozak, M., *J. Mol. Biol.* **196**:947-950 (1987)), a "C" residue to preserve the reading frame, and 16 nucleotides of the sequence of the complete IL-21 protein shown in Figure 1. The 3' primer has the sequence 5'-CGC *GGT ACC* CAC TGA ACG GGG CAG CAC GC-3' (SEQ ID NO:12) containing the *Asp* 718 restriction site followed by 20 nucleotides complementary to the 3' noncoding sequence in Figure 1.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, CA). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, CA).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five µg of a plasmid containing the polynucleotide is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner and colleagues (*Proc. Natl. Acad. Sci. USA* **84**:7413-7417 (1987)). One µg of BaculoGold™ virus DNA and 5 µg of the plasmid are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27°C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith (*supra*). An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200  $\mu$ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5  $\mu$ Ci of  $^{35}$ S-methionine and 5  $\mu$ Ci  $^{35}$ S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced IL-21 protein.

### **Example 8: Expression of IL-21 in Mammalian Cells**

IL-21 polypeptide can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV-I, HIV-1 and the early promoter of the

cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, IL-21 polypeptide can be expressed in stable cell lines containing the IL-21 polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as *dhfr*, *gpt*, neomycin or hygromycin allows the identification and isolation of the transfected cells.

The transfected IL-21 gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest (see, e.g., Alt, F. W., *et al.*, *J. Biol. Chem.* **253**:1357-1370 (1978); Hamlin, J. L. and Ma, C., *Biochem. et Biophys. Acta*, **1097**:107-143 (1990); Page, M. J. and Sydenham, M. A., *Biotechnology* **9**:64-68 (1991)). Another useful selection marker is the enzyme glutamine synthase (GS; Murphy, *et al.*, *Biochem. J.* **227**:277-279 (1991); Bebbington, *et al.*, *Bio/Technology* **10**:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, *et al.*, *Mol. Cell. Biol.*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart, *et al.*, *Cell* **41**:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites *Bam* HI, *Xba* I and *Asp* 718, facilitate the cloning of IL-21. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

IL-21 polynucleotide is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence (see, e.g., WO 96/34891).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC6 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner, et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (for example, 50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100-200  $\mu$ M. Expression of IL-21 is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

#### **Example 9: Protein Fusions of IL-21**

IL-21 polypeptides are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of IL-21 polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification (see Example 5; see also EP A 394,827; Traunecker, *et al.*, *Nature*

331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time *in vivo*. Nuclear localization signals fused to IL-21 polypeptides can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the *Bam* HI cloning site. Note that the 3' *Bam* HI site should be destroyed. Next, the vector containing the human Fc portion is again restricted with *Bam* HI, linearizing the vector, and IL-21 polynucleotide, isolated by the PCR protocol described in Example 1, is ligated into this *Bam* HI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence (see, e.g., WO 96/34891).

#### Human IgG Fc region (SEQ ID NO:13):

```
GGGATCCGGAGCCCAAATCTTCTGACAAACTCACACATGCCACCGTGCCAGCACCTGAATTCGAGGGTGACCCGTCAAGTC
TTCTCTTCCCCCAAAACCAAGGACACCCATGATCTCCCGGACTCTGAGGTACATGCCGTGGTGGACGTAAGCCA
CGAAGACCCGTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGGGGAGGAGCAGT
ACAACAGCACGTACCGTGTGGTCAGCGTCTCTACCGTCTCTGCACCGAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
TCCAACAAAGCCCTCCCAACCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACACAGGTGTACACCCCT
GCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCG
TGGAGTGGGAGAGCAATGGGCACCCGAGAGCAACTACAAGACCAAGCCTCCCGTGGTGGACTCCGACGGCTCTTCTTCTCTC
TACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGCTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGGCAGCGCCGACTCTAGAGGAT
```

#### Example 10: Production of an Antibody



The antibodies of the present invention can be prepared by a variety of methods (see, Current Protocols, Chapter 2). For example, cells expressing IL-21 is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of IL-21 protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler, *et al.*, *Nature* 256:495 (1975); Kohler, *et al.*, *Eur. J. Immunol.* 6:511 (1976); Kohler, *et al.*, *Eur. J. Immunol.* 6:292 (1976); Hammerling, *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with IL-21 polypeptide or, more preferably, with a secreted IL-21 polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands and colleagues (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the IL-21 polypeptide.

Alternatively, additional antibodies capable of binding to IL-21 polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the IL-21 protein-specific antibody can be blocked by IL-21. Such antibodies comprise anti-idiotypic antibodies to the IL-21 protein-specific

antibody and can be used to immunize an animal to induce formation of further IL-21 protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted IL-21 protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For *in vivo* use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art (see, for review, Morrison, *Science* **229**:1202 (1985); Oi, *et al.*, *BioTechniques* **4**:214 (1986); Cabilly, *et al.*, U.S. Patent No. 4,816,567; Taniguchi, *et al.*, EP 171496; Morrison, *et al.*, EP 173494; Neuberger, *et al.*, WO 8601533; Robinson, *et al.*, WO 8702671; Boulianne, *et al.*, *Nature* **312**:643 (1984); Neuberger, *et al.*, *Nature* **314**:268 (1985)).

#### **Example 11: Production Of IL-21 Protein For High-Throughput Screening Assays**

The following protocol produces a supernatant containing IL-21 polypeptide to be tested. This supernatant can then be used in the screening assays described subsequently in Examples 13-20.

First, dilute poly-D-lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (Phosphate Buffered Saline; w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50 µg/ml. Add 200 µl of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate the poly-D-lysine solution and rinse with 1 ml PBS. The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at 2 x 10<sup>5</sup> cells/well in 0.5 ml DMEM (Dulbecco's Modified Eagle Medium) supplemented with 4.5 G/L glucose, L-glutamine (12-604F Biowhittaker), 10% heat inactivated FBS (14-503F Biowhittaker), and 1x Penstrep (17-602E Biowhittaker). Let the cells grow overnight.

Following overnight incubation, mix together in a sterile solution basin: 300  $\mu$ l Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL) in each well of a 96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2  $\mu$ g of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50  $\mu$ l of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT for 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150  $\mu$ l Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by simultaneously performing the following tasks in a staggered fashion. Thus, hands-on time is cut in half, and the cells are not excessively incubated in PBS. First, person A aspirates the media from four 24-well plates of cells, and then person B rinses each well with 0.5-1ml PBS. Person A then aspirates the PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200  $\mu$ l of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Plates are then incubated at 37°C for 6 hours.

While cells are incubating, the appropriate media is prepared: either 1% BSA in DMEM with 1x penstrep, or HGS CHO-5 media (116.6 mg/L of  $\text{CaCl}_2$  (anhyd); 0.00130 mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 0.050 mg/L of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ; 0.417 mg/L of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 311.80 mg/L of KCl; 28.64 mg/L of  $\text{MgCl}_2$ ; 48.84 mg/L of  $\text{MgSO}_4$ ; 6995.50 mg/L of NaCl; 2400.0 mg/L of  $\text{NaHCO}_3$ ; 62.50 mg/L of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 71.02 mg/L of  $\text{Na}_2\text{HPO}_4$ ; .4320 mg/L of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; .002 mg/L of Arachidonic Acid; 1.022 mg/L of Cholesterol; 0.070 mg/L of D-L-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L-Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine- $\text{H}_2\text{O}$ ; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL- $\text{H}_2\text{O}$ ; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL- $\text{H}_2\text{O}$ ; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2 $\text{H}_2\text{O}$ ; and 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate;

11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust osmolarity to 327 mOsm) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 µl for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, again, preferably by two people, at the end of the incubation period. Person A aspirates the transfection media, while person B adds 1.5 ml of the appropriate media to each well. Incubate at 37°C for 45 or 72 hours, depending on the media used (1%BSA for 45 hours or CHO-5 for 72 hours).

On day four, using a 300 µl multichannel pipetter, aliquot 600 µl in one 1ml deep well plate and the remaining supernatant into a 2 ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the IL-21 polypeptide directly (e.g., as a secreted protein) or by IL-21 inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

### **Example 12: Construction of GAS Reporter Construct**

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site ("GAS") elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in

many cell types though it has been found in T-helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

5       The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

10       The Jaks are activated by a wide range of receptors summarized in the Table below (adapted from review by Schidler and Darnell, *Ann. Rev. Biochem.* 64:621-51 (1995)). A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin;  
15       and (b) Class 2 includes IFN- $\alpha$ , IFN- $\gamma$ , and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (where "Xxx" represents any amino acid; SEQ ID NO:14)).

20       Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

25       Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway (see Table below). Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

	<u>Ligand</u>	<u>tyk2</u>	<u>JAKs</u> <u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>	<u>STATS</u>	<u>GAS(elements) or ISRE</u>
<u>IFN family</u>							
5	IFN-a/B	+	+	-	-	1,2,3	ISRE
	IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
	Il-10	+	?	?	-	1,3	
<u>gp130 family</u>							
10	IL-6 (Pleiotrohic)	+	+	+	?	1,3	GAS (IRF1>Lys6>IFP)
	Il-11(Pleiotrohic)	?	+	?	?	1,3	
	OnM(Pleiotrohic)	?	+	+	?	1,3	
	LIF(Pleiotrohic)	?	+	+	?	1,3	
	CNTF(Pleiotrohic)	-/+	+	+	?	1,3	
15	G-CSF(Pleiotrohic)	?	+	?	?	1,3	
	IL-12(Pleiotrohic)	+	-	+	+	1,3	
<u>g-C family</u>							
20	IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
	IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP >>Ly6)(IgH)
	IL-7 (lymphocytes)	-	+	-	+	5	GAS
	IL-9 (lymphocytes)	-	+	-	+	5	GAS
	IL-13 (lymphocyte)	-	+	?	?	6	GAS
25	IL-15	?	+	?	+	5	GAS
<u>gp140 family</u>							
	IL-3 (myeloid)	-	-	+	-	5	GAS (IRF1>IFP>>Ly6)
	IL-5 (myeloid)	-	-	+	-	5	GAS
	GM-CSF (myeloid)	-	-	+	-	5	GAS
30	<u>Growth hormone family</u>						
	GH	?	-	+	-	5	
	PRL	?	+/-	+	-	1,3,5	
	EPO	?	-	+	-	5	GAS(B-CAS>IRF1=IFP>>Ly6)
35	<u>Receptor Tyrosine Kinases</u>						
	EGF	?	+	+	-	1,3	GAS (IRF1)
	PDGF	?	+	+	-	1,3	
	CSF-1	?	+	+	-	1,3	GAS (not IRF1)
40							

To construct a synthetic GAS containing promoter element, which is used in the biological assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS-binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman, *et al.*, *Immunity* 1:457-468 (1994)), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18 bp of sequence complementary to the SV40 early promoter sequence and is flanked with an *Xho* I restriction site. The sequence of the 5' primer is: 5'-GCG CCT CGA GAT TTC CCC GAA ATC TAG ATT TCC CCG AAA TGA TTT CCC CGA AAT GAT TTC CCC GAA ATA TCT GCC ATC TCA ATT AG-3' (SEQ ID NO:15).

The downstream primer is complementary to the SV40 promoter and is flanked with a *Hin* dIII site: 5'-GCG GCA AGC TTT TTG CAA AGC CTA GGC-3' (SEQ ID NO:16).

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with *Xho* I and *Hin* dIII and subcloned into BLSK2- (Stratagene). Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

CTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAGT  
CAGCAACCATAGTCCCGCCCTAACTCCGCCCATCCCGCCCTAACTCCGCCCATCTCCGCCCATCTCCGCCCATGGCTGA  
CTAATTTTATTTATTTATGTCAGAGCCGAGGCCCTCGGCCTCTGAGCTATTCAGAGTAGTGAGGAGGCTTTTGGAGG  
CCTAGGCTTTTGCAAAAAGCTT (SEQ ID NO:17).

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP". Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using *Hin* dIII and *Xho* I, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector

using *Sal* I and *Not* I, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NF- $\kappa$ B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HeLa (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

### **Example 13: High-Throughput Screening Assay for T-cell Activity.**

The following protocol is used to assess T-cell activity of IL-21 by determining whether IL-21 supernatant proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies; transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200  $\mu$ l of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1% Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies)



with 10 µg of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 µl of DMRIE-C and incubate at room temperature for 15-45 min.

During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37°C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with supernatants containing IL-21 polypeptides or IL-21 induced polypeptides as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 µl of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 µl of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 µl samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophane covers) and stored at -20°C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4°C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

#### **Example 14: High-Throughput Screening Assay Identifying Myeloid Activity**

The following protocol is used to assess myeloid activity of IL-21 by determining whether IL-21 proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12.

5 Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda, *et. al.*, *Cell Growth & Differentiation*, 5:259-265 (1994)) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

15 Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8  $\mu$ g GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375  $\mu$ M  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1 mM  $\text{MgCl}_2$ , and 675  $\mu$ M  $\text{CaCl}_2$ . Incubate at 37°C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37°C for 36 hr.

20 The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400  $\mu$ g/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400  $\mu$ g/ml G418 for couple of passages.

These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200  $\mu$ l cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

25 Add 50  $\mu$ l of the supernatant prepared by the protocol described in Example 11. Incubate at 37°C for 48 to 72 hr. As a positive control, 100 U/ml interferon gamma can be used which is known to activate U937 cells. Over 30-fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

### 35 Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed by IL-21.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by IL-21 can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (nucleotides -633 to +1; Sakamoto, K., *et al.*, *Oncogene* 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers: (A) 5' Primer: 5'-GCG CTC GAG GGA TGA CAG CGA TAG AAC CCC GG-3' (SEQ ID NO:18) and (B) 3' Primer: 5'-GCG AAG CTT CGC GAC TCC CCG GAT CCG CCT C-3' (SEQ ID NO:19).

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes *Xho* I and *Hin* dIII, removing the GAS/SV40 stuffer fragment. Digest the EGR1 amplified product with the same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, 2 ml of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 µl per well of the 96-well plate, and allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin on a precoated 10 cm tissue culture dish. A 1:4 split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 µg/ml G418. The G418-free medium is used for routine

growth but every one to two months, the cells should be re-grown in 300 µg/ml G418 for several passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS.

5 Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as 5x10<sup>5</sup> cells/ml.

10 Add 200 µl of the cell suspension to each well of 96-well plate (equivalent to 1x10<sup>5</sup> cells/well). Add 50 µl supernatant produced by Example 11, 37° C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/µl of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

#### **Example 16: High-Throughput Screening Assay for T-cell Activity**

20

NF-κB (Nuclear Factor κB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-α and lymphotoxin-β, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-κB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF-κB appears to shield cells from apoptosis), B- and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

25 In non-stimulated conditions, NF-κB is retained in the cytoplasm with I-κB (Inhibitor κB). However, upon stimulation, I-κB is phosphorylated and degraded, causing NF-κB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF-κB include IL-2, IL-6, GM-CSF, ICAM-1 and class I MHC.

30 Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-κB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-κB would be useful in treating diseases. For example, inhibitors of NF-κB could be used to treat those diseases related to the acute or chronic activation of NF-κB, such as rheumatoid arthritis.

To construct a vector containing the NF- $\kappa$ B promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF- $\kappa$ B binding site (5'-GGG GAC TTT CCC-3'; SEQ ID NO:20), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an *Xho* I site: 5'-GCG GCC TCG AGG GGA CTT TCC CGG GGA CTT TCC GGG GAC TTT CCG GGA CTT TCC ATC CTG CCA TCT CAA TTA G-3' (SEQ ID NO:21).

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a *Hin* dIII site: 5'-GCG GCA AGC TTT TTG CAA AGC CTA GGC-3' (SEQ ID NO:22).

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with *Xho* I and *Hin* dIII and subcloned into BLSK2- (Stratagene). Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5'-CTCGAGGGGACTTTCCCGGGGACTTTCCCGGGGACTTTCCCGGGGACTTTCCATCTGCCATCTCAATTAGTCAGCAACCATAG  
TCCCGCCCTAACTCCGCCCATCCCGCCCTAACTCCGCCAGTTCCGCCCATTTCTCCGCCCATGGCTGACTAATTTT  
ATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTGGAGGCCTAGGCTTTTG  
CAAAAAGCTT-3' (SEQ ID NO:23)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF- $\kappa$ B/SV40 fragment using *Xho* I and *Hin* dIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF- $\kappa$ B/SV40/SEAP cassette is removed from the above NF- $\kappa$ B/SEAP vector using restriction enzymes *Sal* I and *Not* I, and inserted into a vector containing neomycin resistance. Particularly, the NF- $\kappa$ B/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with *Sal* I and *Not* I.

Once NF- $\kappa$ B/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF- $\alpha$  (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

### **Example 17: Assay for SEAP Activity**

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15  $\mu$ l of 2.5x dilution buffer into Optiplates containing 35  $\mu$ l of a supernatant. Seal the plates with a plastic sealer and incubate at 65°C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50  $\mu$ l Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50  $\mu$ l Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

**Table III: Reaction Buffer Formulation:**

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5

33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

#### **Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

5

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

10

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-3, used here.

15

For adherent cells, seed the cells at 10,000-20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 µl of HBSS (Hank's Balanced Salt Solution) leaving 100 µl of buffer after the final wash.

20

A stock solution of 1 mg/ml fluo-3 is made in 10% pluronic acid DMSO. To load the cells with fluo-3, 50 µl of 12 µg/ml fluo-3 is added to each well. The plate is incubated at 37°C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 µl of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to  $2.5 \times 10^6$  cells/ml with HBSS in a 50-ml conical tube. Four  $\mu$ l of 1 mg/ml fluo-3 solution in 10% pluronic acid DMSO is added to each 1 ml of cell suspension. The tube is then placed in a 37°C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to  $1 \times 10^6$  cells/ml, and dispensed into a microplate, 100  $\mu$ l/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200  $\mu$ l, followed by an aspiration step to 100  $\mu$ l final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-3. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50  $\mu$ l. Increased emission at 530 nm indicates an extracellular signaling event caused by the a molecule, either IL-21 or a molecule induced by IL-21, which has resulted in an increase in the intracellular  $\text{Ca}^{2+}$  concentration.

#### **Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., *src*, *yes*, *lck*, *lyn*, *fyn*) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether IL-21 or a molecule induced by IL-21 is capable of



activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4°C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamar Blue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes, treatment with EGF (60ng/ml) or 50 µl of the supernatant produced in Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4°C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and

PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10 µl of 5 µM Biotinylated Peptide, then 10 µl ATP/Mg<sup>2+</sup> (5 mM ATP/50 mM MgCl<sub>2</sub>), then 10 µl of 5x Assay Buffer (40 mM imidazole hydrochloride, pH 7.3, 40 mM β-glycerophosphate, 1 mM EGTA, 100 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5 µl of Sodium Vanadate (1 mM), and then 5 µl of water. Mix the components gently and preincubate the reaction mix at 30°C for 2 min. Initiate the reaction by adding 10 µl of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 µl of 120 mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 µl aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37°C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide.

Wash the MTP module with 300 µl/well of PBS four times. Next add 75 µl of anti-phosphotyrosine antibody conjugated to horse radish peroxidase (anti-P-Tyr-POD (0.5 µl/ml)) to each well and incubate at 37°C for one hour. Wash the well as above.

Next add 100 µl of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 min (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

#### **Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity**

As a potential alternative and/or complement to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1 ml of protein G (1 µg/ml) for 2 hr at room temp (RT). The plates are

then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100 ng/well) against Erk-1 and Erk-2 (1 hr at RT; available from Santa Cruz Biotechnology). To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules. After 3-5 rinses with PBS, the plates are stored at 4°C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6 ng/well) or 50 µl of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10 ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1 µg/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by IL-21 or a molecule induced by IL-21.

#### **Example 21: Method of Determining Alterations in the IL-21 Gene**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art (see, Sambrook, *et al.*, *supra*) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:1. Suggested PCR conditions consist of 35 cycles at 95°C for 30 seconds; 60-120 seconds at 52-58°C; and 60-120 seconds at 70°C, using buffer solutions described by Sidransky and colleagues (*Science* **252**:706 (1991)).

PCR products are then sequenced using primers labeled at the 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase (Epicentre Technologies). The intron-exon borders of selected exons of IL-21 are also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations in IL-21 are then cloned and sequenced to validate the results of the direct sequencing.

PCR products of IL-21 are cloned into T-tailed vectors as described by Holton and Graham (*Nucl. Acids Res.* 19:1156 (1991)) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations in IL-21 not present in unaffected individuals.

5 Genomic rearrangements are also observed as a method of determining alterations in the IL-21 gene. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described by Johnson and coworkers (*Methods Cell Biol.* 35:73-99 (1991)). Hybridization with the labeled probe is carried out using a vast  
10 excess of human cot-1 DNA for specific hybridization to the IL-21 genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera  
15 (Photometrics, Tucson, AZ) and variable excitation wavelength filters (Johnson, C., *et al.*, *Genet. Anal. Tech. Appl.* 8:75 (1991)). Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC). Chromosome alterations of the genomic region of IL-21 (hybridized by the probe) are identified as insertions,  
20 deletions, and translocations. These IL-21 alterations are used as a diagnostic marker for an associated disease.

#### 25 Example 22: Method of Detecting Abnormal Levels of IL-21 in a Biological Sample

IL-21 polypeptides can be detected in a biological sample, and if an increased or decreased level of IL-21 is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one  
30 skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect IL-21 in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies to IL-21, at a final concentration of 0.2 to 10 µg/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example  
35 10. The wells are blocked so that non-specific binding of IL-21 to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing IL-21. Preferably, serial dilutions of the sample should be used to validate

results. The plates are then washed three times with deionized or distilled water to remove unbound IL-21.

Next, 50  $\mu$ l of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature.

- 5 The plates are again washed three times with deionized or distilled water to remove unbound conjugate.

Add 75  $\mu$ l of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot IL-21 polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the IL-21 in the sample using the standard curve.

### 15 **Example 23: Formulating a Polypeptide**

The IL-21 composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the IL-21 polypeptide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of IL-21 administered parenterally per dose will be in the range of about 1  $\mu$ g/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, IL-21 is typically administered at a dose rate of about 1  $\mu$ g/kg/hour to about 50  $\mu$ g/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing IL-21 are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid

filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

5 IL-21 is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U., *et al.*, *Biopolymers* **22**:547-556  
10 (1983)), poly (2- hydroxyethyl methacrylate) (Langer, R., *et al.*, *J. Biomed. Mater. Res.* **15**:167-277 (1981); Langer, R. *Chem. Tech.* **12**:98-105 (1982)), ethylene vinyl acetate (Langer, R., *et al.*) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped IL-21 polypeptides. Liposomes containing the IL-21 are prepared by methods known *per se* (DE 3,218,121;  
15 Epstein, *et al.*, *Proc. Natl. Acad. Sci. USA* **82**:3688-3692 (1985); Hwang, *et al.*, *Proc. Natl. Acad. Sci. USA* **77**:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324). Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30  
20 mol. percent cholesterol, the selected proportion being adjusted for the optimal secreted polypeptide therapy.

For parenteral administration, in one embodiment, IL-21 is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is  
25 non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting IL-21 uniformly and  
30 intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are  
35 also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at

the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

IL-21 is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

IL-21 used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

IL-21 polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous IL-21 polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized IL-21 polypeptide using bacteriostatic Water-For-Injection (WFI).

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, IL-21 may be employed in conjunction with other therapeutic compounds.

#### **Example 24: Method of Treating Decreased Levels of IL-21**

5 The present invention relates to a method for treating an individual in need of a decreased level of IL-21 activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of IL-21 antagonist. Preferred antagonists for use in the present invention are IL-21-specific antibodies.

10 Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of IL-21 in an individual can be treated by administering IL-21, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of IL-21 polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of IL-21 to increase the activity level of IL-21 in such an individual.

15 For example, a patient with decreased levels of IL-21 polypeptide receives a daily dose 0.1-100 µg/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

#### 20 **Example 25: Method of Treating Increased Levels of IL-21**

The present invention also relates to a method for treating an individual in need of an increased level of IL-21 activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of IL-21 or an agonist thereof.

25 Antisense technology is used to inhibit production of IL-21. This technology is one example of a method of decreasing levels of IL-21 polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer.

30 For example, a patient diagnosed with abnormally increased levels of IL-21 is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

#### 35 **Example 26: Method of Treatment Using Gene Therapy**



One method of gene therapy transplants fibroblasts, which are capable of expressing IL-21 polypeptides, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37°C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T., *et al.*, DNA 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with *Eco* RI and *Hin* dIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding IL-21 can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1. Preferably, the 5' primer contains an *Eco* RI site and the 3' primer includes a *Hin* dIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified *Eco* RI and *Hin* dIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector contains properly inserted IL-21.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the IL-21 gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the IL-21 gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media



*What Is Claimed Is:*

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polynucleotide fragment of SEQ ID NO:1 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: 209666;
- (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (c) a polynucleotide encoding conserved polypeptide domain I of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (d) a polynucleotide encoding conserved polypeptide domain II of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (e) a polynucleotide encoding conserved polypeptide domain III of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (f) a polynucleotide encoding conserved polypeptide domain IV of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (g) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (h) a polynucleotide encoding a polypeptide of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666 having biological activity;
- (i) a polynucleotide which is a variant of SEQ ID NO:1;
- (j) a polynucleotide which is an allelic variant of SEQ ID NO:1;
- (k) a polynucleotide which encodes a species homologue of the polypeptide whose amino acid sequence is shown in SEQ ID NO:2;
- (l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j) or (k), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues; and
- (m) a polynucleotide which is the complement of any one of the polynucleotides specified in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k) or (m).

2. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polynucleotide fragment of SEQ ID NO:28;
- (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:28;
- (c) a polynucleotide encoding conserved polypeptide domain I of SEQ ID NO:28;
- (d) a polynucleotide encoding conserved polypeptide domain II of SEQ ID NO:28;
- (e) a polynucleotide encoding conserved polypeptide domain III of SEQ ID NO:28;
- (f) a polynucleotide encoding conserved polypeptide domain IV of SEQ ID NO:28;
- (g) a polynucleotide encoding conserved polypeptide domain V of SEQ ID NO:28;
- (h) a polynucleotide encoding conserved polypeptide domain VI of SEQ ID NO:28;
- (i) a polynucleotide encoding conserved polypeptide domain VII of SEQ ID NO:28;
- (j) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:28;
- (k) a polynucleotide encoding a polypeptide of SEQ ID NO:28 having biological activity;
- (l) a polynucleotide which is a variant of SEQ ID NO:28;
- (m) a polynucleotide which is an allelic variant of SEQ ID NO:28;
- (n) a polynucleotide which encodes a species homologue of the polypeptide whose amino acid sequence is shown in SEQ ID NO:28;
- (o) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m) or (n), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues; and
- (p) a polynucleotide which is the complement of any one of the polynucleotides specified in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n) or (o).

3. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:



6. The isolated nucleic acid molecule of claim 3, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a mature form or a secreted protein.

7. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:2 or the coding sequence included in ATCC Deposit No: 209666.

8. The isolated nucleic acid molecule of claim 2, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:28.

9. The isolated nucleic acid molecule of claim 3, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:4 or the coding sequence included in ATCC Deposit No: 209665.

10. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:1 or the cDNA sequence included in ATCC Deposit No: 209666.

11. The isolated nucleic acid molecule of claim 2, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:28.

12. The isolated nucleic acid molecule of claim 3, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:3 or the cDNA sequence included in ATCC Deposit No: 209665.

13. The isolated nucleic acid molecule of claims 4, 5 or 6, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

14. The isolated nucleic acid molecule of claims 7, 8 or 9, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

15. A recombinant vector comprising the isolated nucleic acid molecule of claims 1, 2 or 3.

16. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

17. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 2.

18. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 3.

19. A recombinant host cell produced by the method of claim 16.

20. A recombinant host cell produced by the method of claim 17.

21. A recombinant host cell produced by the method of claim 18.

22. The recombinant host cell of claim 19 comprising vector sequences.

23. The recombinant host cell of claim 20 comprising vector sequences.

24. The recombinant host cell of claim 21 comprising vector sequences.

25. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666;

(b) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666 having biological activity;

(c) a polypeptide domain of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666;

(d) a polypeptide epitope of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666;

(e) a mature form of a secreted protein;

(f) a full length secreted protein;

(g) a variant of SEQ ID NO:2;

- (h) an allelic variant of SEQ ID NO:2; and
- (i) a species homologue of the SEQ ID NO:2.

26. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polypeptide fragment of SEQ ID NO:29;
- (b) a polypeptide fragment of SEQ ID NO:29 having biological activity;
- (c) a polypeptide domain of SEQ ID NO:29;
- (d) a polypeptide epitope of SEQ ID NO:29;
- (e) a mature form of a secreted protein of SEQ ID NO:29;
- (f) a full length secreted protein of SEQ ID NO:29;
- (g) a variant of SEQ ID NO:29;
- (h) an allelic variant of SEQ ID NO:29; and
- (i) a species homologue of the SEQ ID NO:29.

27. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polypeptide fragment of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665;
- (b) a polypeptide fragment of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665 having biological activity;
- (c) a polypeptide domain of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665;
- (d) a polypeptide epitope of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665;
- (e) a mature form of a secreted protein;
- (f) a full length secreted protein;
- (g) a variant of SEQ ID NO:4;
- (h) an allelic variant of SEQ ID NO:4; and
- (i) a species homologue of the SEQ ID NO:4.

28. The isolated polypeptide of claims 26, 26 or 27, wherein the mature form or the full length secreted protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.



29. An isolated antibody that binds specifically to the isolated polypeptide of claims 25, 26 or 27.

30. A recombinant host cell that expresses the isolated polypeptide of claim 25.

31. A recombinant host cell that expresses the isolated polypeptide of claim 26.

32. A recombinant host cell that expresses the isolated polypeptide of claim 27.

33. A method of making an isolated polypeptide comprising:  
(a) culturing the recombinant host cell of claim 30 under conditions such that said polypeptide is expressed; and  
(b) recovering said polypeptide.

34. A method of making an isolated polypeptide comprising:  
(a) culturing the recombinant host cell of claim 31 under conditions such that said polypeptide is expressed; and  
(b) recovering said polypeptide.

35. A method of making an isolated polypeptide comprising:  
(a) culturing the recombinant host cell of claim 32 under conditions such that said polypeptide is expressed; and  
(b) recovering said polypeptide.

36. The polypeptide produced by claims 33, 34 or 35.

37. A method for preventing, treating, or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of the polypeptide of claims 25, 26 or 27 or of the polynucleotide of claims 1, 2 or 3.

38. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to expression or activity of a secreted protein comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claims 1, 2 or 3;

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

39. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to expression or activity of a secreted protein comprising:

(a) determining the presence or amount of expression of the polypeptide of claims 25, 26 or 27 in a biological sample;

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

40. A method for identifying binding partner to the polypeptide of claims 25, 26 or 27 comprising:

(a) contacting the polypeptide of claims 25, 26 or 27 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

41. The gene corresponding to the cDNA sequence of SEQ ID NO:1.

42. The gene corresponding to the cDNA sequence of SEQ ID NO:28.

43. The gene corresponding to the cDNA sequence of SEQ ID NO:3.

44. A method of identifying an activity in a biological assay; wherein the method comprises:

(a) expressing SEQ ID NO:1 in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

45. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:28 in a cell;

- (b) isolating the supernatant;
- (c) detecting an activity in a biological assay; and
- (d) identifying the protein in the supernatant having the activity.

46. A method of identifying an activity in a biological assay, wherein the method comprises:

- (a) expressing SEQ ID NO:3 in a cell;
- (b) isolating the supernatant;
- (c) detecting an activity in a biological assay; and
- (d) identifying the protein in the supernatant having the activity.

47. The product produced by the method of claim 44.

48. The product produced by the method of claim 45.

49. The product produced by the method of claim 46.

## Abstract

5 The present invention relates to novel human proteins designated Interleukin-21 (IL-21) and Interleukin-22 (IL-22), and isolated polynucleotides encoding these proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human proteins.

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 ggnttcggtc ggcgactctg aagagagtnc accgagcaaa ccaagtgccg gagcaacagc 240  
 gncgnctttt ncatggagat tcgtaagcan ttttcatttg acanggggat ccctgggttg 300  
 tttttagtta caagcaagca nntggnttga agtngntggg gaaaggancc gnagggattc 360  
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Leu Gln Ser Leu Leu Val Leu Arg Arg Arg Pro Cys Ser Arg Asp Gly			
155	160	165	
tcg ggg ctc ccc aca cct ggg gcc ttt gcc ttc cac acc gag ttc atc			582
Ser Gly Leu Pro Thr Pro Gly Ala Phe Ala Phe His Thr Glu Phe Ile			
170	175	180	
cac gtc ccc gtc ggc tgc acc tgc gtg ctg ccc cgt tca gtg			624
His Val Pro Val Gly Cys Thr Cys Val Leu Pro Arg Ser Val			
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Thr Pro His Cys Tyr Ser Ala Glu Glu Leu Pro Leu Gly Gln Ala Pro			
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Pro His Leu Leu Ala Arg Gly Ala Lys Trp Gly Gln Ala Leu Pro Val			
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Ala Leu Val Ser Ser Leu Glu Ala Ala Ser His Arg Gly Arg His Glu			
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Arg Pro Ser Ala Thr Thr Gln Cys Pro Val Leu Arg Pro Glu Glu Val			
85	90	95	
Leu Glu Ala Asp Thr His Gln Arg Ser Ile Ser Pro Trp Arg Tyr Arg			

100	105	110
Val Asp Thr Asp Glu Asp Arg Tyr Pro Gln Lys Leu Ala Phe Ala Glu		
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Cys Leu Cys Arg Gly Cys Ile Asp Ala Arg Thr Gly Arg Glu Thr Ala		
130	135	140
Ala Leu Asn Ser Val Arg Leu Leu Gln Ser Leu Leu Val Leu Arg Arg		
145	150	155
Arg Pro Cys Ser Arg Asp Gly Ser Gly Leu Pro Thr Pro Gly Ala Phe		
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agagcctgac tgggtgctgcg ccgcccggccc tgactaccg cnacggacta cgggggctac 180  
cccacacctg gggncctttg accttcaca ccgnagttac atgccacgta ccccgttcgg 240  
gctgtcacct gacgtgctgn ccccgtttac agtgtgnacc gaccgtaggn ccgtggggtc 300  
ccctnagtac tggnacacgt gtgatacccc ag 332

60099905-041095



Figure 1  
Interleukin-21

```

1  GGCACGAGTGGACACGGATGAGGACCGCTATCCACAGAAGCTGGCCTTCGCCGAGTGCCT  60
1  A R V D T D E D R Y P Q K L A F A E C L  20
      Domain I                               Domain II

61  GTGCAGAGGCTGTATCGATGCACGGACGGGCCGCGAGACAGCTGCGCTCAACTCCGTGCG  120
21  C R G C I D A R T G R E T A A L N S V R  40
      Domain II

121 GCTGCTCCAGAGCCTGCTGGTGTGCGCCGCCGCCCTGCTCCCGCGACGGCTCGGGGCT  180
41  L L Q S L L V L R R R P C S R D G S G L  60
      Domain III

181 CCCCACACCTGGGGCCTTTGCCCTTCCACACCGAGTTTCATCCACGTCCCCGTGGGCTGCAC  240
61  P T P G A F A F H T E F I H V P V G C T  80
      Domain IV

241 CTGCGTGTGCCCCGTTCAGTGTGACCGCCAAGGCCGTGGGGCCTTAGACTGGACACGT  300
81  C V L P R S V  87
      Domain IV

301 GTGCTCCCCAGAGGGCACCCCTATTATTATGTGTATTATTATTATTATATGCCCTCCCC  360

361 AACACTACCCCTTGGGGTCTGGGCATTCCCCGTGTCTGGAGGACAGCCCCCACTGTTCTC  420

421 CTCACTCCAGCCTCAGTAGTTGGGGGTWGAAGGAGCTCAGCACCTCTTCCAGCCCTTAA  480

481 AGCTGCAGAAAAGGTGTACACGGCTGCCTGTACCTTGGYTCCCTGTCTGCTCCCGGCT  540

541 TCCCTTACCTTATCACTGGCCTCAGGCCCGCCGAGGCTGCCTCTTCCCAACCTCCTTGA  600

601 AGTACCCCTGTTTCTTAAACAATTATTAAAGTGTACGTGTATTATTAAACTGATGAACAC  660

661 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  705

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Figure 2A  
Interleukin-22

```

1  GGAATTCGGCACGAGCTCGTGCCGTGCTCAGTGCCCTTCCACCACACGCTGCAGCTGGGGC  60
1  N S A R A R A V L S A F H H T L Q L G P  20

      #

61  CGCGTGAGCAGGCGCGCAACGCGAGCTGCCCGGCAGGGGGCAGGCCCGCGACCGCGCT  120
21  R E Q A R N A S C P A G G R P A D R R F  40

121  TCCGGCCGCCACCAACCTGCGCAGCGTGTCGCCCTGGGGCTACAGAATCTCCTACGACC  180
41  R P P T N L R S V S P W A Y R I S Y D P  60
                                   Domain I

181  CGGCGAGGTACCCCAGGTACCTGCCTGAAGCCTACTGCCTGTGCCGGGGCTGCCTGACCG  240
61  A R Y P R Y L P E A Y C L C R G C L T G  80
    Domain I                               Domain II

241  GGCTGTTTCGGCGAGGAGGACGTGCGCTTCCGCAGCGCCCTGTCTACATGCCCAACCGTCG  300
81  L F G E E D V R F R S A P V Y M P T V V  100
                                   Domain III

301  TCCTGCGCCGCACCCCGCCTGCGCCGGCGCGCGTTCGGTCTACACCGAGGCCCTACGTCA  360
101  L R R T P A C A G G R S V Y T E A Y V T  120
    Domain III

      #

361  CCATCCCGTGGGCTGCACCTGCGTCCCCGAGCCGGAGAAGGACCGCAGACAGCATCAACT  420
121  I P V G C T C V P E P E K D A D S I N S  140
    Domain IV

421  CCAGCATCGACAAACAGGGCGCAAGCTCCTGTGCTGGGCCCAACGACGCGCCCGCTGGCC  480
141  S I D K Q G A K L L L G P N D A P A G P  160

481  CCTGAGGCGCGTTCCTGCCCGGGAGGTCTCCCGGGCCCGCATCCCGAGGCGCCCAAGCTG  540

541  GAGCGCCTGGAGGGCTCGGTTCGGCGACCTCTGAAGAGAGTGACCGAGCAAACCAAGTG  600

601  CCGGAGCACCAGCGCGCCTTTCCATGGGACTCGTAAGCAGCTTCATCTGACACGGGCA  660

661  TCCCTGGCTTGCTTTTAGCTACAAGCAAGCAGCGTGGCTGGAAGCTGATGGGAAACGACC  720

721  CGGCACGGGCATCCTGTGTGCGGCCCGCATGGAGGGTTTGGAAAAGTTCACGGAGGCTCC  780

781  CTGAGGAGCCTCTCAGATCGGCTGCTGCGGGTGCAGGGCGTGACTCACCCTGGGTGCTT  840

841  GCCAAAGAGATAGGGACGCATATGCTTTTAAAGCAATCTAAAAATAATAAAGTATAG  900

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Figure 2B  
Interleukin-22

901 CGACTATATACCTACTTTTAAATCAACTGTTTTGAATAGAGGCAGAGCTATTTTATATT 960  
961 ATCAAATGAGAGCTACTCTGTTACATTTCTTAACATATAAACATCGTTTTTTACTTCTTC 1020  
1021 TGGTAGAATTTTTTAAAGCATAATTGGAATCCTTGGATAAATTTGTAGCTGGTACACTC 1080  
1081 TGGCCTGGGTCTCTGAATTCAGCCTGTCACCGATGGCTGACTGATGAAATGGACACGTCT 1140  
1141 CATCTGACCCACTCTTCCTTCCACTGAAGGTCTTCACGGGCCTCCAGGTGGACCAAAGGG 1200  
1201 ATGCACAGGCGGCTCGCATGCCCCAGGGCCAGCTAAGAGTTCCAAAGATCTCAGATTGG 1260  
1261 TTTTAGTCATGAATACATAAACAGTCTCAAACTCGCACAATTTTTTCCCCCTTTTGAAAG 1320  
1321 CCACTGGGGCCAATTTGTGGTTAAGAGGTGGTGAGATAAGAAGTGGAACGTGACATCTTT 1380  
1381 GCCAGTTGTCAGAAGAATCCAAGCAGGTATTGGCTTAGTTGTAAGGGCTTTAGGATCAGG 1440  
1441 CTGAATATGAGGACAAAGTGGGCCACGTTAGCATCTGCAGAGATCAATCTGGAGGCTTCT 1500  
1501 GTTCTGCAATCTGCCACGAGAGCTAGGTCCTTGATCTTTCTTTAGATTGAAAGTCTGT 1560  
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1621 GCTGAAAAAAAAAAAAAAAAA 1642

550T50"03.1093

Figure 3A

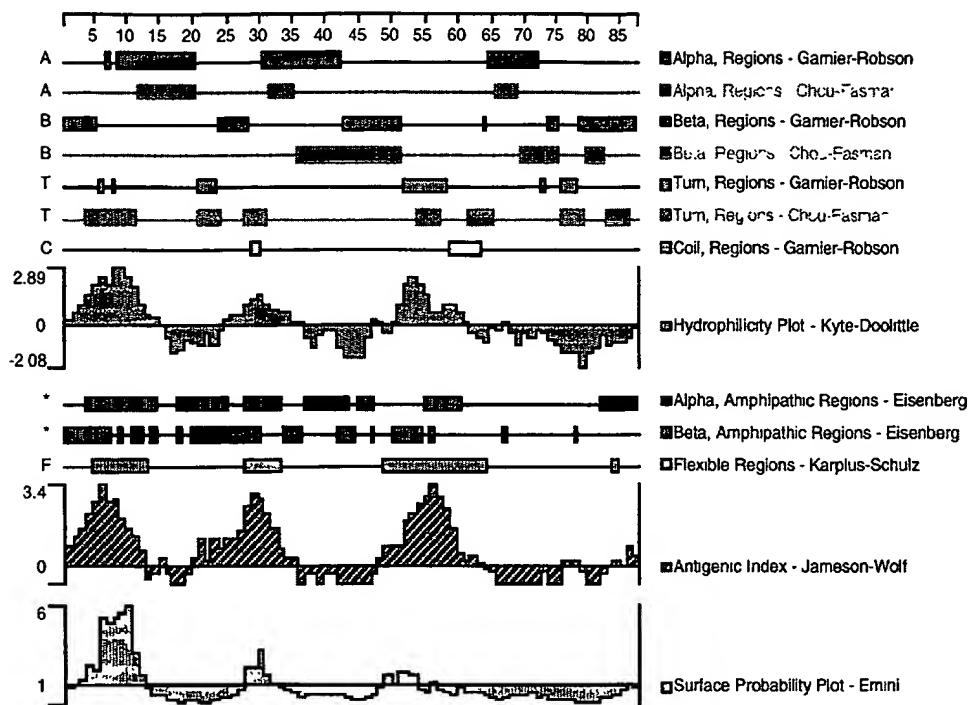
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1	MSPGRASSVS	L	-	AAIAIPQSSAC	PNTEA	KDFF	mIL-17.aa
1	MTFRMTSLV	-	-	CCIVKSEITSAQT	PRCLAN	-	vIL-17.aa
1	MDWPHNLLFL	L	-	-	-	-	IL20.aa
1	A	-	-	-	-	-	IL-21.aa
1	NS	-	-	-	-	-	IL-22.aa
1	MTLLPGLLFL	TWLTCL	AHHDPSLRGHPHSHGTP	HCYSAE	ELPLGQA	PH	IL21FL.aa
		60	70	80	90	100	
48	NLNI	-	-	-	-	-	IL-17.aa
50	NLKV	FNSLGAKV	-	-	-	-	mIL-17.aa
46	TLSI	-	-	-	-	-	vIL-17.aa
37	PLAPGP	-	-	-	-	-	IL20.aa
2	-	-	-	-	-	-	IL-21.aa
22	EQARN	-	-	-	-	-	IL-22.aa
51	LARGA	KWGQALPV	ALVSSLEAASHRGH	ERP	PSAT	TCQVLRPEEVL	IL21FL.aa
		110	120	130	140	150	
69	-	-	-	-	-	-	IL-17.aa
72	-	-	-	-	-	-	mIL-17.aa
65	-	-	-	-	-	-	vIL-17.aa
84	CEVN	LQLWMS	NKRS	LSPW	GYSIN	HDP	IL20.aa
2	-	-	-	-	-	-	IL-21.aa
44	-	-	-	-	-	-	IL-22.aa
101	-	-	-	-	-	-	IL21FL.aa

Figure 3B

	160	170	180	190	200	
106	V D Y H M N S V P I Q Q E I L V L R R E - - - - -	P P H C P N S F R L E K I L V S V G C T C	IL-17.aa			
109	L D H M N S V L I Q Q E I L V L K R E - - - - -	P E S C P F T F R V E K M L V G V G C T C	mIL-17.aa			
102	V D Y H M N S V P I Q Q E I L V V R K G - - - - -	H Q P C P N S F R L E K M L V T V G C T C	vIL-17.aa			
134	E D R S M V S V P V F - S Q V P V R R R L C P P P P R T G P C R Q - - -	R A V M E T I A V G C T C	IL20.aa			
32	E T A L N S V R L L Q S L L V L R R R P C S R D G S G L P T P G A F A F H T E F I H V P V G C T C	IL-21.aa				
85	E D V R F S A P V Y M P T V V L R R R T P A C A G G R S V - - - - -	Y T E A Y V T I P V G C T C	IL-22.aa			
142	E T A A L N S V R L L Q S L L V L R R R P C S R D G S G L P T P G A F A F H T E F I H V P V G C T C	IL21FL.aa				
	210	220	230			
147	V T P I V H H V A				IL-17.aa	
150	V A S I V R Q A A				mIL-17.aa	
143	V T P I V H N V D				vIL-17.aa	
179	I - - - - -				IL20.aa	
82	V - - - - -				IL-21.aa	
128	V P E P E K D A D S I N S S I D K Q G A K L L G P N D A P A G P				IL-22.aa	
192	V - - - - -				IL21FL.aa	

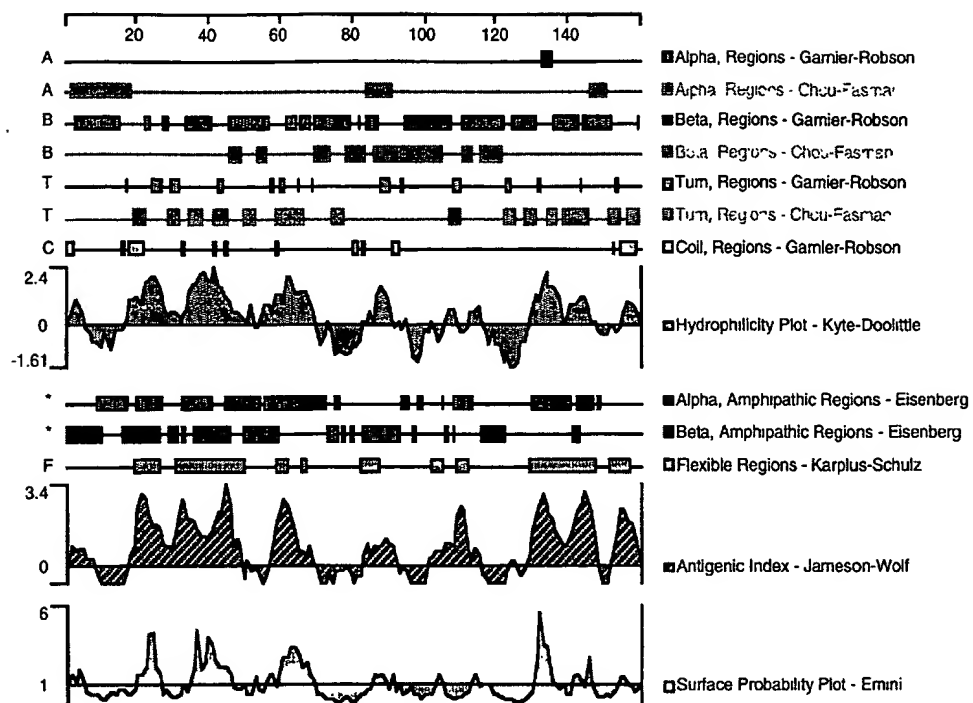
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Figure 4  
Interleukin-21 Polypeptide Analysis



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Figure 5  
Interleukin-22 Polypeptide Analysis



60099805 "04.1.058

Figure 6A  
Interleukin-21

1 GCTCCAAGCCAGCCTGCCCCGCTGCGCCACCATGACGCTCCTCCCGGCTCCTGTTT 60  
1 M T L L P G L L F 9

61 CTGACCTGGCTGCACACATGCCTGGCCCAACATGACCCCTCCCTCAGGGGGCACCCCCAC 120  
10 L T W L H T C L A H H D P S L R G H P H 29

121 AGTCACGGTACCCACACTGCTACTCGGCTGAGGAAGTGGCCCTCGGCCAGGCCCCCCCA 180  
30 S H G T P H C Y S A E E L P L G Q A P P 49  
Domain V

181 CACCTGCTGGCTCGAGGTGCCAAGTGGGGGAGGCTTTGCCTGTAGCCCTGGTGTCCAGC 240  
50 H L L A R G A K W G Q A L P V A L V S S 69  
Domain VI

241 CTGGAGGCAGCAAGCCACAGGGGGAGGCACGAGAGGCCCTCAGCTACGACCCAGTGCCCG 300  
70 L E A A S H R G R H E R P S A T T Q C P 89

301 GTGCTGCGGCCGAGGAGGTGTGGAGGCAGACCCACAGCGCTCCATCTCACCCCTGG 360  
90 V L R P E E V L E A D T H Q R S I S P W 109  
Domain VII

361 AGATACCGGTGGACACGGATGAGGACCGCTATCCACAGAAGCTGGCCTTCGCCGAGTGC 420  
110 R Y R V D T D E D R Y P Q K L A F A E C 129  
Domain I Domain II

421 CTGTGCAGAGGCTGTATCGATGCACGGACGGGCGGAGACAGCTGCGCTCAACTCCGTG 480  
130 L C R G C I D A R T G R E T A A L N S V 149  
Domain II

481 CGGCTGCTCCAGAGCCTGCTGGTGCTGCGCGCGCGGCCCTGCTCCCGCGACGGCTCGGGG 540  
150 R L L Q S L L V L R R R P C S R D G S G 169  
Domain III

541 CTCCCCACACCTGGGGCCTTTGCCTTCCACACGAGTTTCATCCACGTCCCGCTCGGCTGC 600  
170 L P T P G A F A F H T E F I H V P V G C 189  
Domain IV

601 ACCTGCGTGCTGCCCCGTTCAAGTGTGACCGCCAAGGCCGTGGGGCCCTTAGACTGGACAC 660  
190 T C V L P R S V 197  
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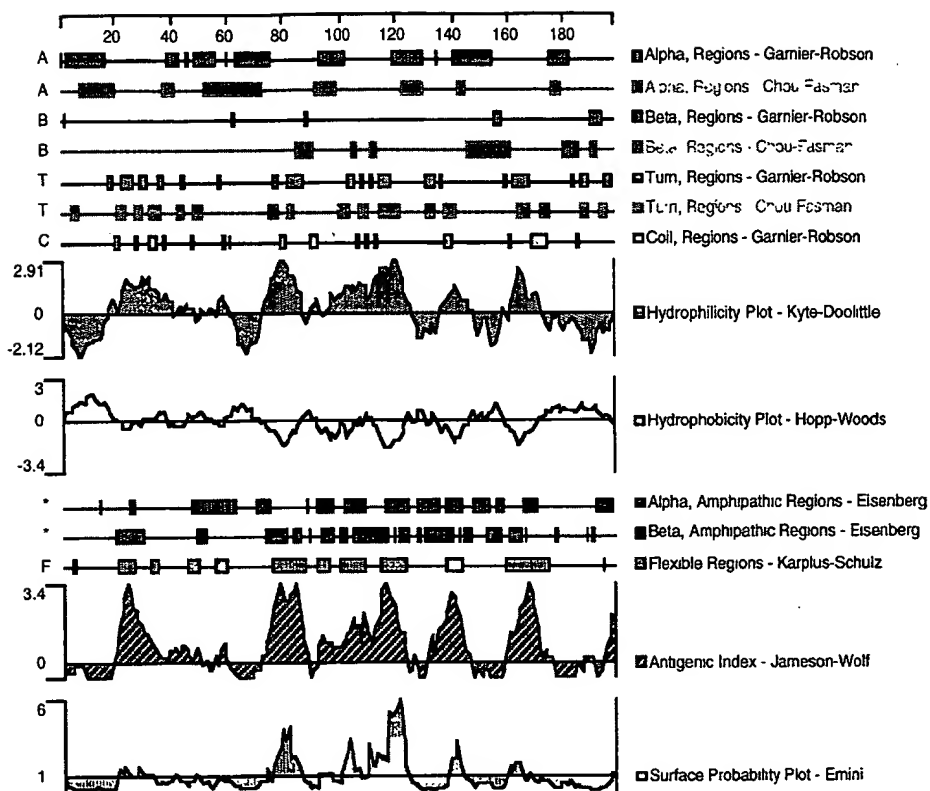
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60099305-091095



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Figure 7  
Interleukin-21 Polypeptide Analysis



360705-091003

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